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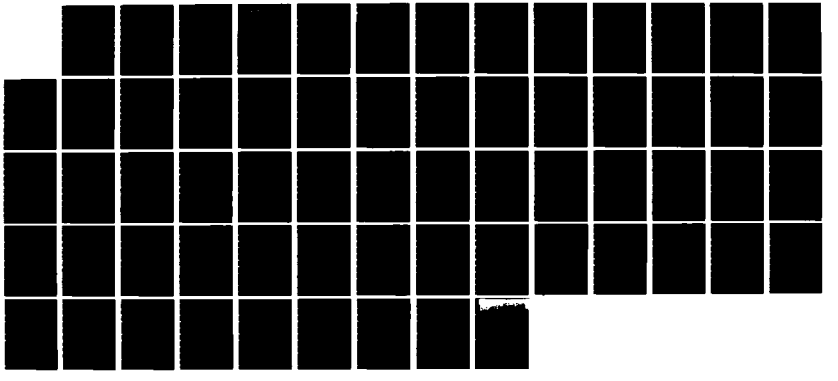

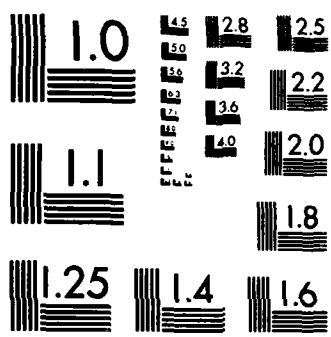

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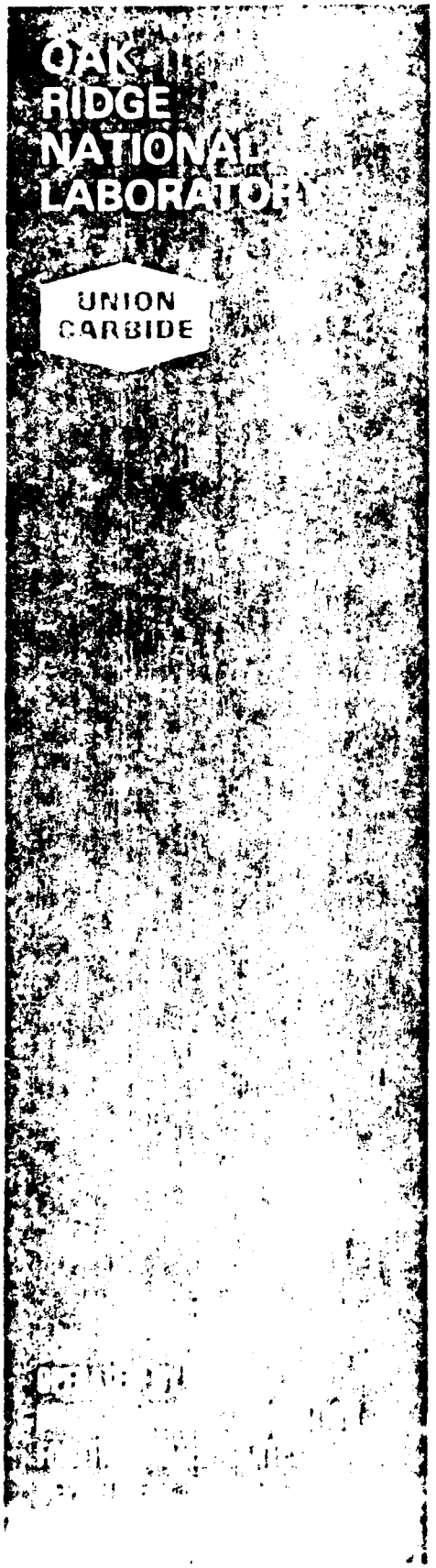

Chemical Characterization and Toxicologic Evaluation of Airborne Mixtures

Chemical Characterization of Army Colored Smokes: Inventory Smoke Mixes (Red, Violet, Yellow, and Green)

FINAL REPORT

I. B. Rubin
M. V. Buchanan

JUNE 1983

Supported by
U.S. ARMY MEDICAL RESEARCH
AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, MD 21701

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ARY NOTES

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one; 1,4-Diaminoanthraquinone; 1,4-Diamino-2,3-dihydroanthraquinone; rysenedione; Differential Solubility; Gas Chromatography; Gas Chroma- Mass Spectroscopy; Green Smoke Mix; Infrared Spectroscopy; Liquid raphy; Mass Spectroscopy; 1-Methylaminoanthraquinone; Nuclear Resonance Spectroscopy; Particle Size Distribution; Red Smoke Mix;

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et, yellow and green inventory smoke mixes were fractionated by blimation, differential solubility and liquid chromatography. Two mponents, benzanthrone and dibenzochrysenedione were purified by the ods. Major components of the smoke mixes were isolated and identi- omparison to the pure dyes using a variety of instrumental tech- A number of contaminants at very minor levels were identified by gas raphy/mass spectroscopy. All of the smoke mixes contained relatively ntities (10-25 percent) of chloroform insoluble or non-volatile

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19. Signal Smokes; Spectrophotometry; Spectroscopy; Thin Layer Chromatography; 1,4-di-p-toluidinoanthraquinone; Vacuum Sublimation; Violet Smoke Mix; Yellow Smoke Mix

20. undifferentiated carbonaceous material,

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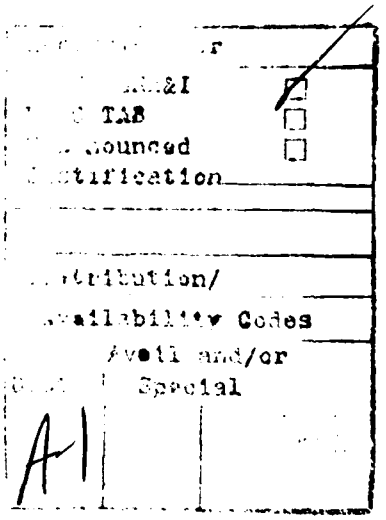
CHEMICAL CHARACTERIZATION OF ARMY COLORED SMOKES: INVENTORY SMOKE MIXES (RED, VIOLET, YELLOW, AND GREEN)

FINAL REPORT

I. B. Rubin and M. V. Buchanan

Bio/Organic Analysis Section
Analytical Chemistry Division

JUNE 1983



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Project Officers: Mary C. Henry, Ph.D., James C. Eaton
Health Effects Research Division
U. S. ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT LABORATORY
Fort Detrick, Frederick, MD 21701

EXECUTIVE SUMMARY

As part of a project to identify potential health effects of U. S. Army smoke materials, four colored smoke mixes used in the M18 smoke grenade have been separated and characterized. A combination of vacuum sublimation, differential solubility, and liquid chromatograhy was used to effect the separations. Identification of the smoke mix components was done by various means dependent upon the solubility and volatility of the major components. Thin layer chromatography, gas chromatography, combined gas chromatography/mass spectrometry, nuclear magnetic resonance spectroscopy (^{1}H and ^{13}C) and ultraviolet/visible, and fluorescence spectrometry were employed to identify compounds that were sufficiently soluble or volatile for these techniques. For those compounds that were not amenable to these techniques, direct probe mass spectrometry and solid phase nuclear magnetic resonance were used.

The red smoke mix (RSM) was found to contain primarily 1-methylaminoanthraquinone (MAA), as expected. Two to three percent of anthraquinone was also observed in the RSM, along with trace amounts of other materials. The most unusual feature of the RSM was the presence of about 13 percent of a dark, high molecular weight material which was neither soluble nor volatile under normal conditions and thus, could not be fully characterized. This same type of residual material was found in all four smoke mixes.

The yellow smoke mix (YSM), was found to contain benzanthrone (BZA) and dibenzochrysenedione (DBC) primarily, but significant amounts of a ketone-like compound and a series of alkanes were also detected. The alkanes are thought to be from a commercial antidusting agent which is commonly added to DBC. The DBC was found to be virtually insoluble in most common organic solvents and thus, sulfuric acid was used as the solvent for NMR studies. The insolubility of DBC, along with its low volatility, precluded the use of gas chromatographic techniques for the identification of trace impurities. A significant portion (22 percent) of the YSM was a non-volatile residue.

The green smoke mix (GSM) was found to contain BZA, DBC, plus 1,4-di-p-toluidinoanthraquinone (PTA). Several alkanes were observed in the GSM, as in the case of the YSM, and these are thought to arise from the antidusting agent in the DBC dye mix. Several other compounds were observed but could not be identified by direct probe mass spectrometry.

The violet smoke mix (VSM) was one of the more interesting smoke mixes studied. This mix is a yellowish brown in color, as is its major component, 1,4-diamino-2,3-dihydroanthraquinone (DDA). During the separation and characterization of this smoke mix, however, the color of the material was observed to change to a brilliant purple. Spectroscopic studies showed that the DDA is readily converted to 1,4-diaminoanthraquinone (DAA) in air. Because the extent of this oxidation process changed with sample handling conditions, the amounts of DDA and DAA in the various fractions could not be readily determined. Other materials identified in the VSM include significant amounts of 1-methylaminoanthraquinone, a high molecular weight, insoluble residue, and a number of other organics present at trace levels.

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INTRODUCTION

The U. S. Army uses colored smokes, disseminated via the M18 grenade, for signalling and marking, and in some cases, in training to simulate chemical agents. Because of the potential for exposure of workers and instructional cadre and trainees to the smoke fill materials, the U. S. Army Medical Research and Development Command has supported research to study the potential health effects of the colored smoke materials. This report describes work done as part of that effort. The fill materials used in the current inventory smoke grenades were chemically and physically characterized and separated into fractions for assay of their potential for mutagenic activity.

A total of four smoke mixes (red, yellow, green and violet, obtained from Pine Bluff Arsenal, Pine Bluff, AK, have been separated and characterized. The major components of each have been identified and have been sent to the Biology Division for bioassay using several short-term genetic assays. In addition, five dye standards which are used in the formulation of the four smoke mixes have been obtained from The U. S. Army Chemical Research & Development Center, Aberdeen Proving Ground, MD. These dyes have been used to scope various separation schemes and to serve as chromatographic and spectroscopic standards. Additionally, the particle size distributions of the four smoke mixes have been determined.

The chemical analysis of dyestuffs up to about 1950 was reviewed by Venkataraman[1], including a discussion of the various types of chromatographic separations available up to 1950. It was summarized that the separation of two or more dyes from each other is much more difficult than the separation of a dye either from inorganic materials or from non-dye organic materials. Advances in the field of chromatography since that time have led to vast improvements in the separations of dyestuffs[2,3]. However, for the preparative scale separation of the colored smoke mixes undertaken in this study, standard chromatographic techniques alone were not sufficient. The low solubility of some of the dye components in the commonly used chromatographic solvents made the chromatographic separation of the whole smoke mix all but impossible. However, this solubility problem did give rise to the possibility of separation of the dyes by differential solubility techniques. Additionally, the necessity of recovering all materials separated in the smoke mixes made the use of differential solubility and vacuum sublimation attractive alternatives to standard chromatographic techniques.

A combination of vacuum sublimation, differential solubility, and liquid chromatography was used in the separation of the four smoke mixes. The progress of the separations was monitored primarily by thin layer chromatography (TLC). In these TLC studies, eight different types of adsorbent layers were tested with twelve different solvent systems to establish the best separation for each smoke mix and dye. The details of these separation methods are outlined in the following section.

Identification of the smoke mix components also proved to be challenging due to the low solubilities of these compounds. When the compounds were sufficiently soluble and/or volatile, analyses were carried out using thin layer chromatography (TLC), gas chromatography (both packed and capillary column), combined gas chromatography/mass spectrometry (GC/MS) and spectroscopic techniques such as nuclear magnetic resonance (NMR), infrared (IR), ultraviolet and visible (UV/VIS), and fluorescence. However, some materials were sufficiently insoluble and/or non-volatile that techniques such as direct probe mass spectrometry and solid phase NMR were required for the analyses of these materials. The results from these last two techniques were, in general, complex and not as easily interpreted as more conventional analyses. When possible, the dye components were identified by comparison of their spectra with those of pure dyes. For those components not having standards available, their structure was deduced from a combination of IR, NMR, and mass spectra. Finally, the particle size distributions of the four smoke mixes were determined and the results from these studies are given in the following section.

MATERIALS AND METHODS

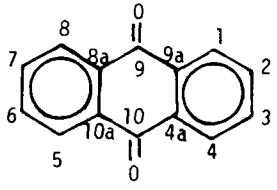
SOURCES OF DYE MATERIALS

A set of anthraquinone derived dye standards was supplied by the U.S. Army Armament Research and Develoment Command, Aberdeen Proving Ground, Maryland, courtesy of R. G. Grafton, Chief, Chemical Systems Division, Product Assurance Directorate. This set included:

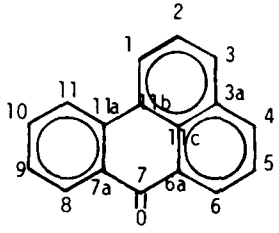
(1) Benzanthrone (BZA, Fig 1 II) [7H-benz(d,e)anthracen-7-one, CAS Registry No. 82-05-3];
(2) 1-methylaminoanthraquinone (MAA, Fig 1 III) [1(methylamino)-9,10-anthracenedione, CAS 82-38-2] [CI Disperse Red 9, CI Solvent Red 111, CI Constitution No. 60505];
(3) 1,4-di-p-toluidinoanthraquinone (PTA, Fig. 1 IV) [1,4-bis-[4-(methylphenyl)-amino]-9,10-anthracenedione, CAS 128-80-3] [CI Solvent Green 3, 61565];
(4) 1,4-diamino-2,3-dihydroanthraquinone (DDA, Fig. 1 V) [1,4-diamino-2,3-dihydro-9,10-anthracenedione, CAS 81-63-0] and
(5) dibenzochrysenedione (DBC, Fig. 1 VII) [dibenzo(b,def)chrysene-7,14-dione, CAS 128-66-5] [CI Vat Yellow 4, 59100].

In addition, 1,4-diaminoanthraquinone (DAA, Fig. 1 VI), [1,4-diamino-9,10-anthracenedione, CAS 128-95-0] [CI Disperse Violet 1, CI Solvent Violet 11, 61100] was prepared from DDA by heating a dimethylsulfoxide solution of DDA (10 mg/ml) at 90°C overnight, or by separating it from a chloroform solution of DDA using thin layer chromatography.

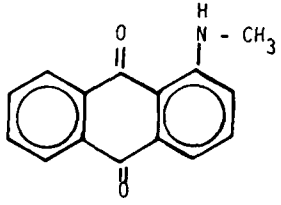
Some commercial dye compounds were obtained to scope possible fractionation and identification techniques prior to the receipt of the



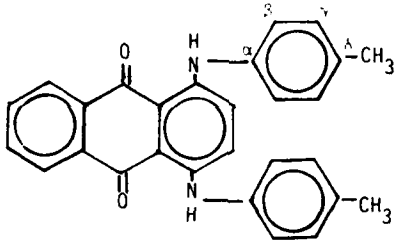
I
Anthracene-9,10-dione (Anthraquinone)



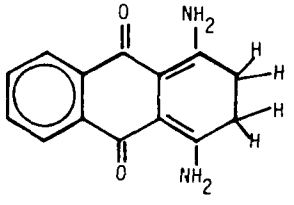
II
7H-benz(d,e)anthracen-7-one
(BZA)



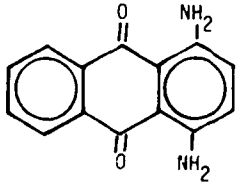
III
1-(methylamino)-9,10-anthracenedione
(MAA)



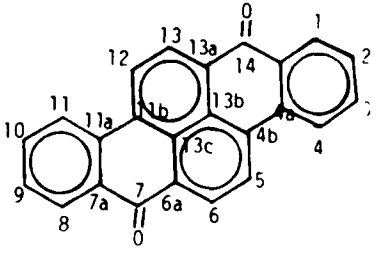
IV
1,4,-di-p-toluidino-9,10-anthracenedione
(PTA)



V
1,4-diamino-2,3-dihydro-9,10-anthracenedione
(DDA)



VI
1,4-diamino-9,10-anthracenedione
(DAA)



VII
dibenzo[b,def]chrysene-7,14-dione
(DBC)

Figure 1. Structural Formulas and Nomenclature for the Colored Smoke Dyes

dye samples and smoke mixes. Benzanthrone and 1,4-diamino-2,3-dihydroanthraquinone were purchased from Pfaltz and Bauer, Inc.; 1-methylaminoanthraquinone was purchased from Aldrich Chemical Co., Inc., and anthraquinone (ANT, Fig. 1 I) [9,10-anthracenedione, CAS 84-65-1] was obtained from Matheson, Coleman and Bell.

Four colored smoke mixes of the types used in the M18 signal grenades were provided from Pine Bluff Arsenal through CPT C. D. Rowlett, U. S. Army Medical Bioengineering Research and Development Laboratory. The four dye mixes (1 kg each) were received in plastic bags and transferred to glass containers. Samples of each were sent to the Biology Division, Oak Ridge National Laboratory, for mutagenicity studies of the whole smoke mixes. The four smoke mixes and their indicated composition of dyes were (1) Red Smoke Mix (RSM), 100 percent MAA; (2) Yellow Smoke Mix (YSM), 64 percent BZA and 36 percent DBC; (3) Green Smoke Mix (GSM), 70 percent PTA, 20 percent BZA and 10 percent DBC; and (4) Violet Smoke Mix (VSM), 80 percent DDA and 20 percent MAA.[4]

SEPARATIONS

A variety of separation methods was used to isolate the components of the four smoke mixes, depending upon the solubilities and volatilities of the dye components. Soxhlet extractions were performed with extraction thimbles obtained from Schleicher & Schuell, Inc. (No. 603). The thimbles were extracted overnight with hot chloroform prior to use. BioRad AG10 basic alumina was used in the column chromatographic separations. Separation by vacuum sublimation was accomplished using a standard sublimation apparatus immersed in a heated oil bath. The lowered pressure (75 mtorr) was obtained with a rotary vacuum pump. The volatile fractions were collected at the indicated temperatures for five hours and were washed off the water-cooled condenser with distilled solvents.

Analytical scale thin layer chromatography (TLC) was the primary method used for monitoring the effectiveness of the separatory procedures. Both Polygram SIL-G (0.25 mm) and SIL-N-HR (0.20 mm) silica plates (obtained from Brinkmann Instruments, Inc.) were used, with equivalent results. The red and violet smoke mixes and their individual components were chromatographed with a methylethylketone/chloroform/acetic acid (80/60/1) developing solution.[5] The green and yellow smoke mixes and their individual constituents were chromatographed with a cyclohexane/diethylether (1/1) developing solution.[6] Fluorescent bands were detected under a 366 nm light source. The TLC data for the six major smoke mix components are given in Table 1.

In some cases where components were sufficiently volatile (primarily for the red and violet smoke mixes), packed column gas chromatography was also used for monitoring the separations procedures. A Perkin-Elmer Model 3920 gas chromatograph with the standard flame ionization detector was used. Chromatography was accomplished on a 12 ft.

TABLE 1. THIN LAYER CHROMATOGRAPHIC CHARACTERISTICS OF COLORED SMOKE DYES ON SILICA[a]

Dye	R_f	Color	
		Visible	Fluorescent
MAA[b]	0.58	Red	Dark
DDA[b]	0.21	Yellow	Bright Yellow
DAA[b]	0.42	Purple	Dark Red
BZA[c]	0.38	Yellow	Light Blue
DBC[c]	0	Orange-Brown	Red-Orange
PTA[c]	0.51	Blue	Dark

[a]Machery-Nagel SIL-N-HR, 0.2 mm.

[b]Methylethylketone/chloroform/acetic acid (80/60/1).

[c]Cyclohexane/diethyl ether (1/1).

by 1/8 in. O.D. glass column packed with 3 percent Dexsil 400 on 80/100 mesh Chromosorb W. Various temperature programs were applied, depending upon the particular sample.

Specific details of the separation procedures used for each of the standard dyes and smoke mixes are outlined below.

Dye Standards

The purities of the dye standards were checked using UV/VIS spectrophotometry at the wavelengths listed in Table 2 and thin layer chromatography, using the conditions listed in Table 1. The MAA, PTA, and DDA were found to be essentially pure (greater than 98 percent). The BZA standard received was reported to be nearly 99 percent pure. However, a substantial amount of chloroform insoluble (19.7 percent) and/or non-volatile (19.3 percent at 245°C and 75 mtorr) material was found in this standard. The BZA was separated into four fractions using vacuum sublimation as shown in Figure 2.

The DBC standard dye was stated to be about 80 percent pure. A separation scheme for DBC is shown in Figure 3. The DBC was first separated by vacuum sublimation into volatiles (fraction 1) and non-volatiles. The non-volatile fraction was then extracted in a Soxhlet apparatus using chloroform, and the nonsoluble residue was labelled fraction 4. The soluble portion was dried and weighed and then washed with two 20 mL aliquots of chloroform. The soluble material was identified as fraction 2 and the insolubles as fraction 3.

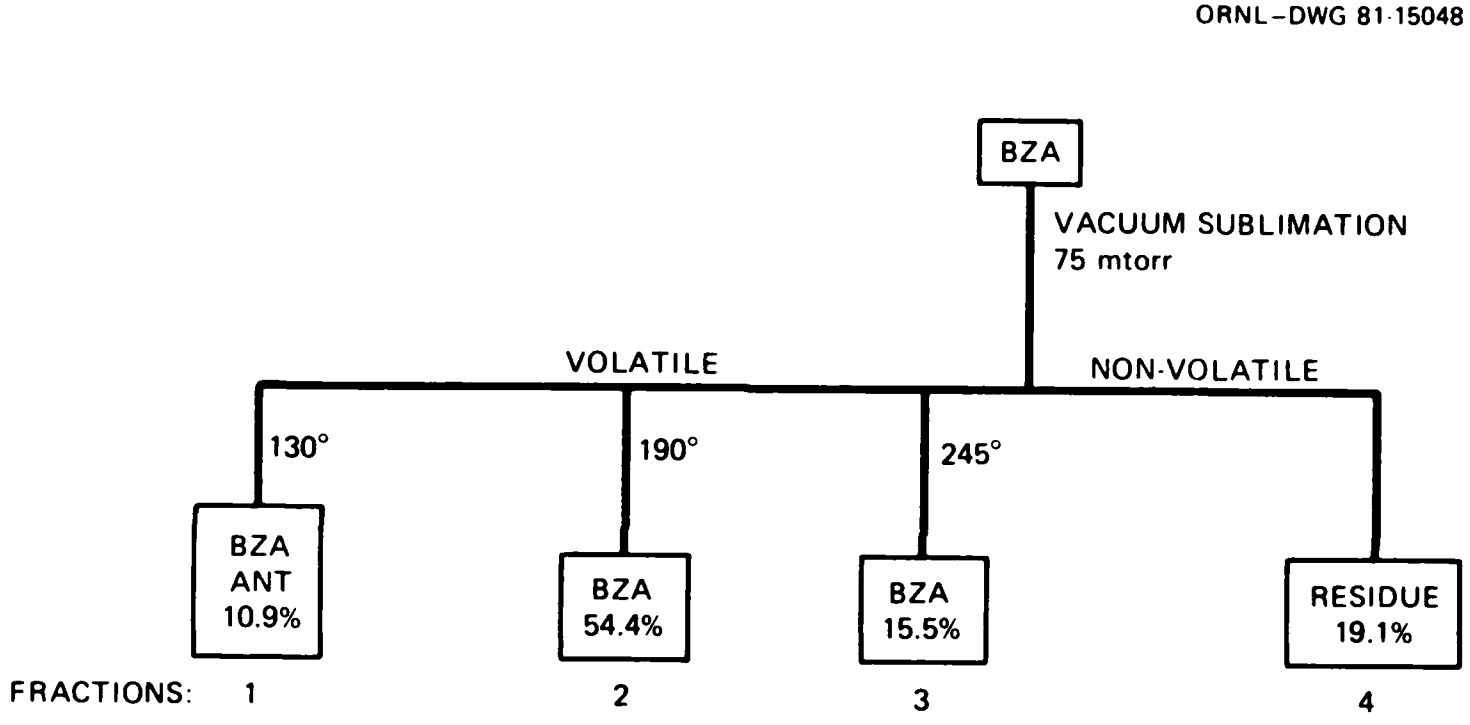


Figure 2. Separation Flowsheet for Benzanthrone (BZA)

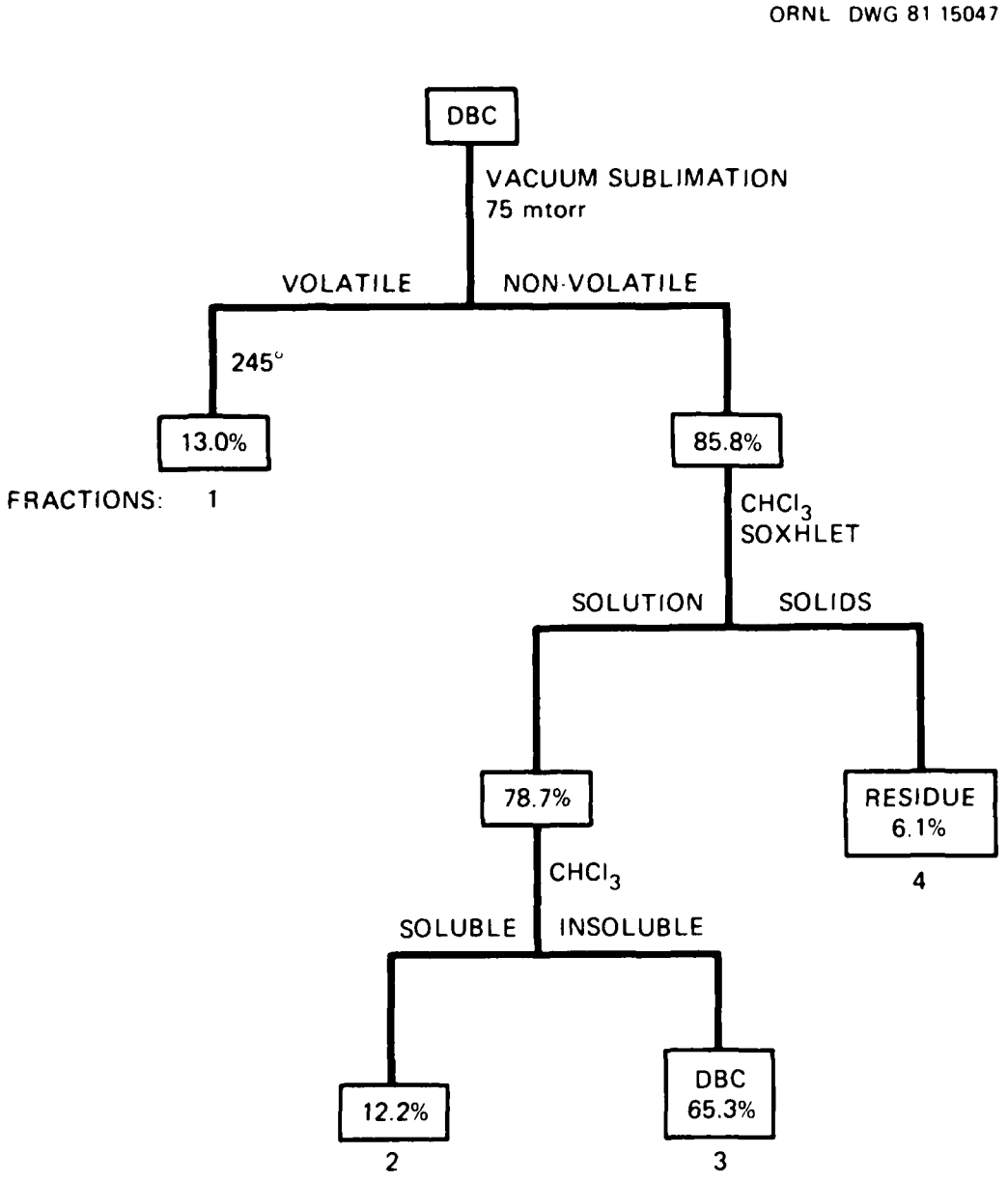


Figure 3. Separation Flowsheet for Dibenzochrysenedione (DBC)

TABLE 2. SPECTRAL CHARACTERISTICS OF COLORED SMOKE DYES IN CHLOROFORM

Dye	λmax, nm			Molar Absorbancy Index x 10^{3a}	
	UV	VIS	Fluor	UV	VIS
BZA	238	392	453	38.55	15.45
DBC	238	465	477	56.88	20.44
DDA	253	456	504	24.36	11.46
DAA	250	545	456	34.46	11.19
MAA	246	508	598	55.72	10.26
PTA	254	645	460	45.54	23.40

[a]Calculated:(7)

$$a_m = \frac{A_s M}{cb}$$

where: A_s = absorbance
M = molecular weight
c = concentration, g/1000 g
b = length of light path, cm

Smoke Mixes

Red Smoke Mix (RSM). The RSM was separated into three fractions by vacuum sublimation, as shown in Figure 4. The major components of the three fractions are given as well as the percent of the original material recovered in each fraction.

Violet Smoke Mix (VSM). The separation of the VSM into four fractions, as shown in Figure 5, was based upon the difference in solubilities of the two major components in chloroform. The sample was first equilibrated in chloroform for one hour and then filtered through a 4.5-5.5 μm glass frit. The solvent was removed from the material in solution and the recovered material was weighed and then transferred to another fine-fritted glass funnel. This material was then washed through the funnel with two 10 mL aliquots of chloroform. The soluble material was labelled fraction 1 and the insolubles fraction 2. The dried solids from the initial separation in chloroform (77.3 percent of the starting material) were transferred to an extraction thimble and extracted continuously with hot chloroform until no further color was observed in the extract. The first portions of this extract were purple (indicating the presence of the more soluble DAA as explained later). The later portions of this extract were yellow (indicating the presence of the less soluble DDA). The soluble material from this extraction was labelled as fraction 3 and the insolubles as fraction 4.

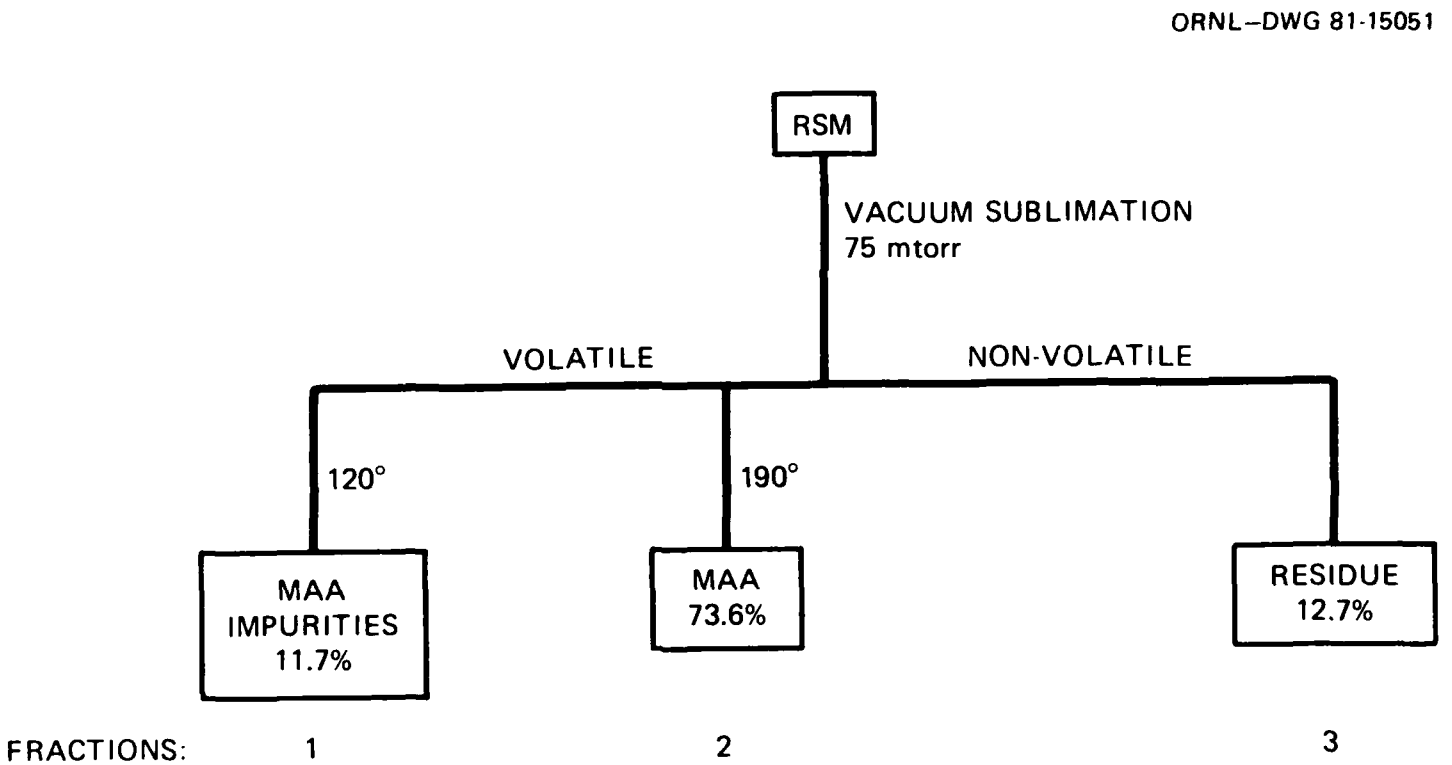


Figure 4. Separation Flowsheet for Red Smoke Mix (RSM)

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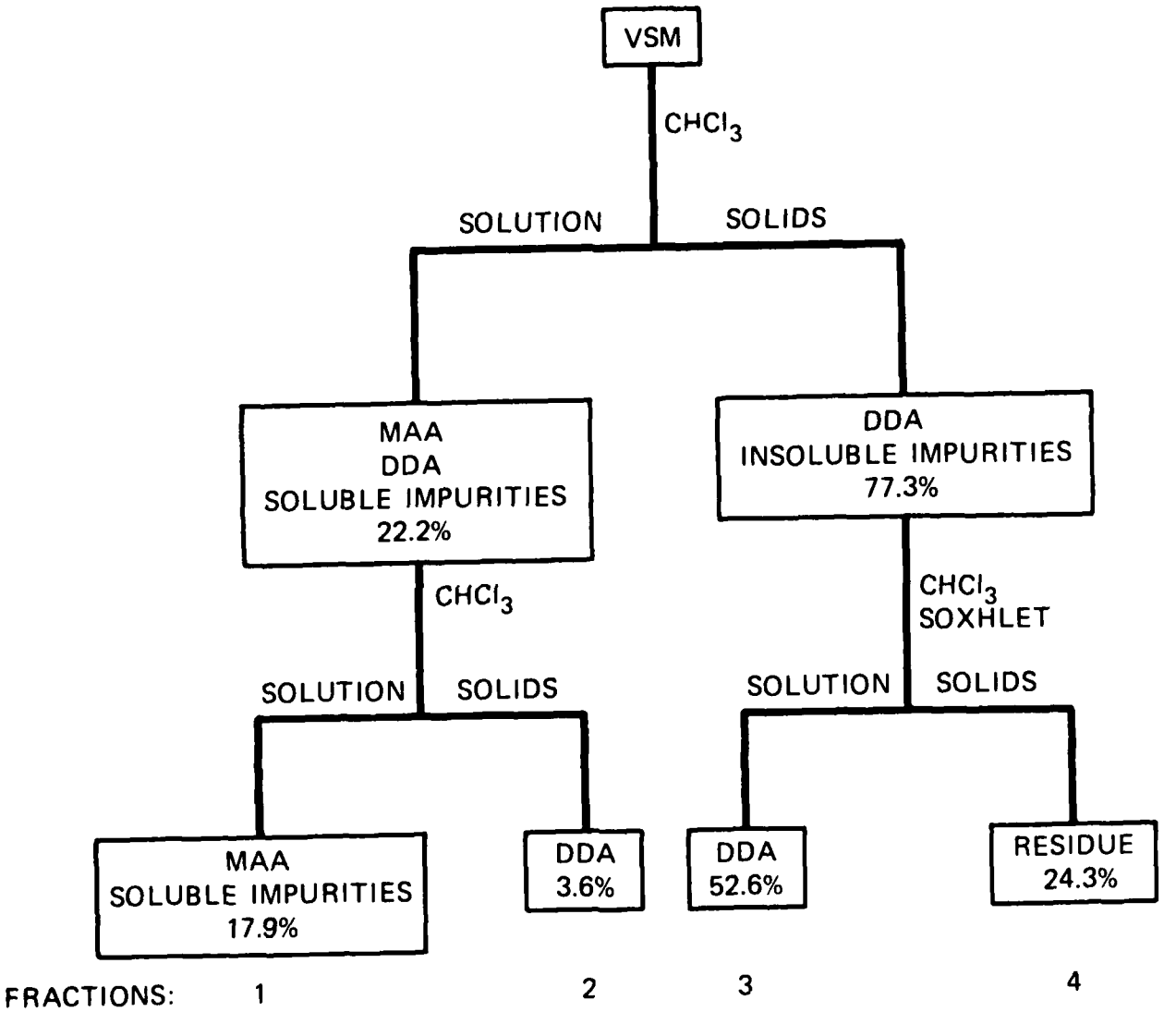

Figure 5. Separation Flowsheet for Violet Smoke Mix (VSM)

Yellow Smoke Mix (YSM). The separation of YSM into seven fractions, as shown in Figure 6, was accomplished using a combination of vacuum sublimation and differential solubility. The sample was initially subjected to vacuum sublimation at 75 mtorr, with the four volatile fractions (fractions 1-4) collected at the temperatures shown on the flow chart. The non-volatile portion (47.7 percent) was transferred to an extraction thimble and extracted continuously with hot chloroform until no color was noted in the extract. The soluble portion was dried, weighed, and then transferred to a fine-fritted glass funnel, where it was washed with two 20 mL portions of chloroform and filtered. The chloroform soluble material was labelled fraction 5 and the nonsoluble material was labelled fraction 6. The solids recovered from the Soxhlet extraction (21.6 percent) were labelled fraction 7.

Green Smoke Mix (GSM). The separation of the GSM was the most difficult of the four smoke mixes studied. This is because BZA and PTA, the major components of GSM, have similar solubility properties and sublime together over a wide temperature range. Therefore, column chromatography was used to help separate these materials. The GSM was separated into five fractions as shown in Figure 7. The GSM was first extracted in a Soxhlet apparatus using hot chloroform until the extract was colorless. The insoluble material was labelled fraction 1. The soluble material was dried and weighed and then separated on a basic alumina column. This separation was accomplished on a 3.8 cm OD x 90 cm column packed with approximately 750 g of basic alumina in cyclohexane. An aliquot of 0.6 to 0.8 g of the soluble material in chloroform was first mixed with 25 to 30 g of the basic alumina. The solvent was removed and the "dyed" alumina was poured onto the head of the column. The column was then developed in chloroform. The collection of fraction 2 began when the first noticeable color appeared in the eluate and continued until the color changed from blue to green. This required approximately 2.7 L of chloroform. The second cut (18.5 percent) was eluted with additional chloroform (about 350 mL) until no further coloration was detected. The second chloroform cut was separated further, using vacuum sublimation, into volatiles (fraction 3) and non-volatiles (fraction 4). After the second cut, the column was washed with two to four liters of methanol. This eluate was labelled fraction 5. A bright blue band along with some pink material was retained at the head of the column and the entire column was a light blue color even after elution with four liters of methanol. This material could not be removed by volatilization (up to 245°C at 100 mtorr). Recovery of the GSM was only 93 percent, compared to 98 percent or better for the other colored smoke mixes, as shown in Table 3.

Spectroscopic Methods

Visible and ultraviolet spectra were obtained using 1 cm cell in a Cary Model 14 double beam spectrophotometer. Chloroform was used as the solvent in all cases, although DDA and DAA were also studied in DMSO. All samples were run at concentrations of 0.02 mg/mL for visible spectra and from 0.005 to 0.01 mg/mL for ultraviolet spectra, as noted.

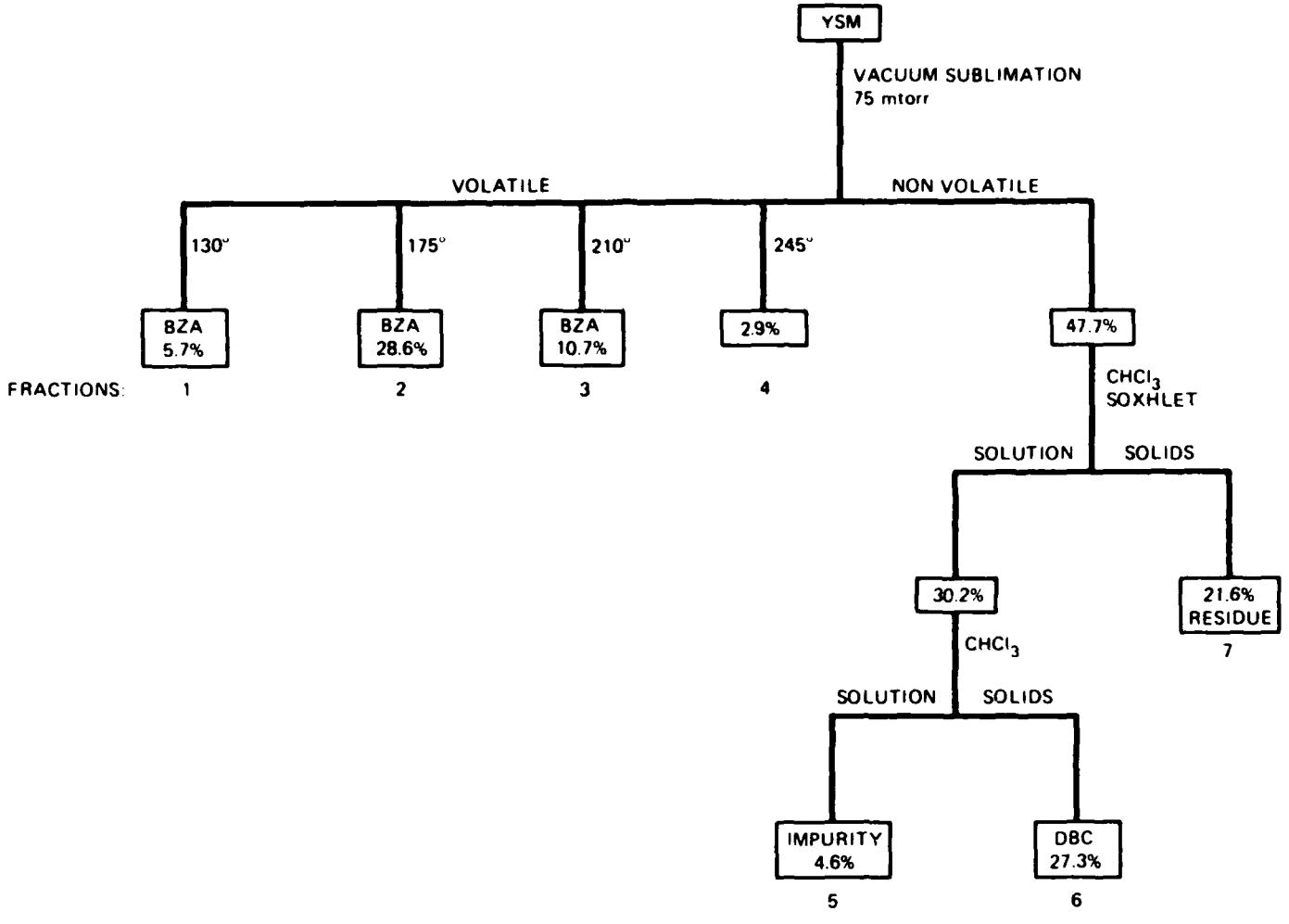

Figure 6. Separation Flowsheet for Yellow Smoke Mix (YSM)

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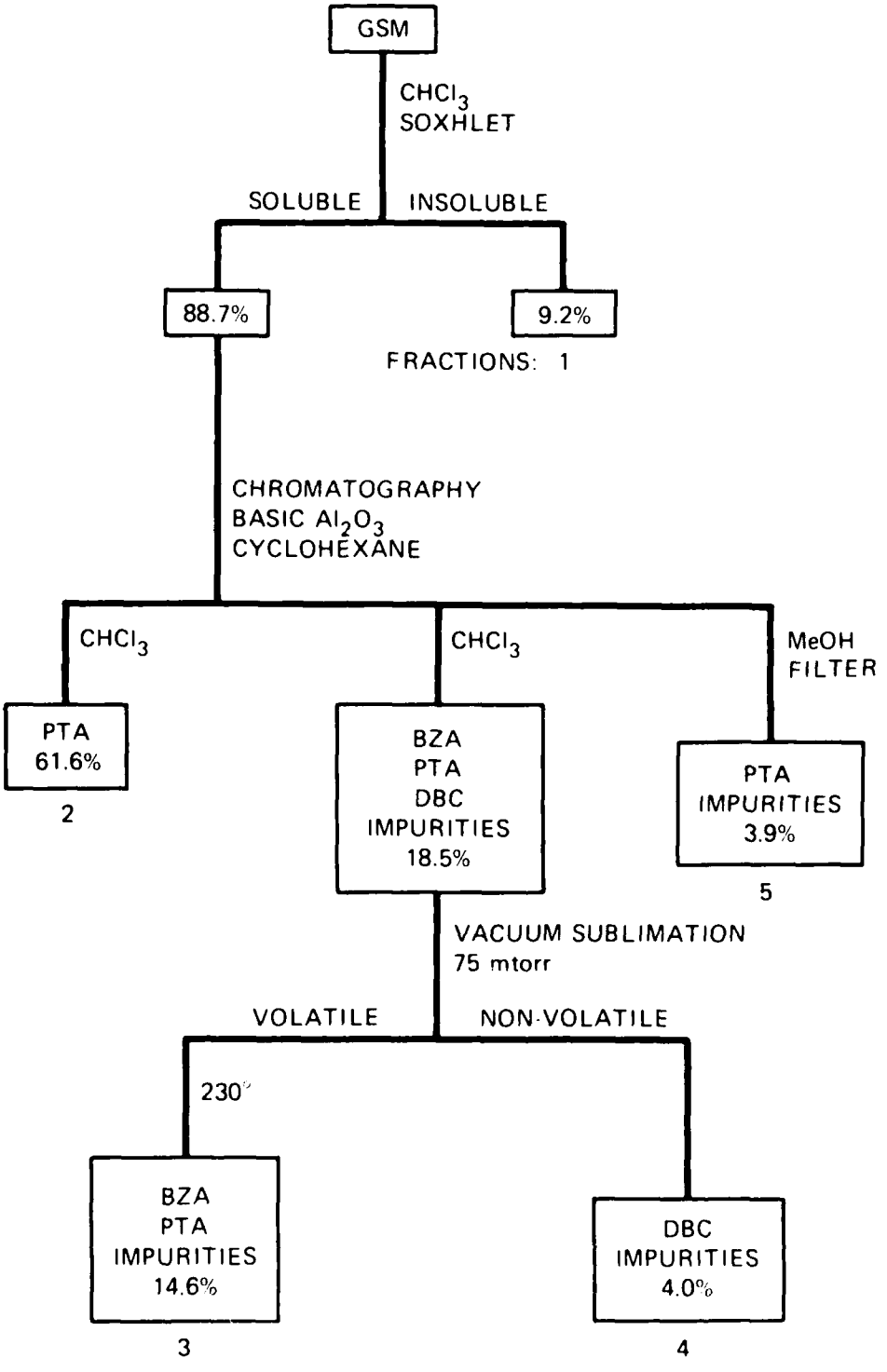

Figure 7. Separation Flowsheet for Green Smoke Mix (GSM)

TABLE 3. REPRODUCIBILITY OF SEPARATION PROCEDURES FOR COLORED SMOKE MIXES

		Portion of Whole Sample, %			
	Trial	1	2	$\bar{x}$	S.E.$_R$[a]
Red Smoke Mix					
Fraction 1		12.95	10.43	11.69	
2		73.39	73.77	73.58	
3		12.77	12.55	12.66	
	Total	99.11	96.75	97.93	16
Violet Smoke Mix					
Fraction 1		18.07	17.64	17.86	
2		3.88	3.36	3.62	
3		52.58	52.57	52.58	
4		25.60	22.96	24.28	
	Total	100.13	96.35	98.24	9
Yellow Smoke Mix					
Fraction 1		5.98	5.45	5.72	
2		31.76	25.43	28.60	
3		6.76	14.59	10.68	
4		4.48	1.34	2.91	
5		4.35	4.77	4.56	
6		25.19	29.48	27.34	
7		18.80	24.41	21.61	
	Total	97.32	104.84	101.08	5
Green Smoke Mix					
Fraction 1		5.41	13.06	9.24	
2		62.16	61.00	61.58	
3		15.57	13.64	14.61	
4		3.22	4.79	4.01	
5		3.14	3.67	3.41	
	Total	89.50	96.16	92.83	42

[a]Relative standard error at 95% confidence level, based on difference between duplicates.

Fluorescence spectra were run using a Perkin-Elmer Model MPF-44A fluorescence spectrophotometer with sample concentrations of 0.01 mg/mL in chloroform. The UV, visible, and fluorescence spectral parameters for the six major components are given in Table 2.

Infrared spectra were obtained using a Digilab FTS-20C Fourier transform infrared spectrometer. Samples were prepared as potassium bromide pellets using about 1 mg of sample per 300 mg pellet. A blank pellet was used as the reference, and spectra were accumulated for 100 scans.

Proton (^{1}H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were obtained at 89.55 MHz and 22.50 MHz, respectively, using a JEOL FX90Q Fourier transform NMR spectrometer. The spectra were run at an ambient probe temperature of 30°C, and with an internal deuterium lock. The samples were run in deuterated chloroform or dimethylsulfoxide with tetramethylsilane (TMS) added as an internal reference. The proton spectra were run using a 28 sec (90°) pulse, a 2.5 second pulse delay, and four pulse accumulation. The ^{13}C spectra were generally run using a 12 sec (45°) pulse and 15 second pulse delay. The number of scans accumulated for the ^{13}C spectra varied with each sample, but most spectra were run overnight ($\sim$ 4500 scans) due to the low solubilities of the compounds.

Mass spectra were obtained with a Hewlett-Packard 5985A gas chromatograph/mass spectrometer (GC/MS), with the electron impact source at 70 eV. The non-volatile samples were introduced into the mass spectrometer via a direct insertion probe which was temperature programmed from room temperature to 200°C. Volatile samples were introduced via a gas chromatograph interfaced to the mass spectrometer. The gas chromatograph was equipped with a fused silica capillary column coated with OV-101 (SGE, Austin, TX) or SE-52 (J&W Scientific, Orangval, CA). Helium was used as the carrier gas at 30 lbs. head pressure. The inlet and GC/MS transfer lines were maintained at 280°C and the oven temperature was programmed from 80 to 280°C at 3°C/min. A splitless injector was used to introduce the sample.

Elemental Analysis

Carbon and hydrogen were determined using a Leco C-H analyzer. Nitrogen was determined by micro-Kjeldahl and oxygen by neutron activation.

Particle Size Distribution

The particle size distributions of the four dye mixtures were measured using a Bahco Microparticle Classifier. Approximately 12 g of each of the dye powders was dried overnight at 105-110°C. After cooling in a dessicator, the sample was weighed on an analytical balance

and then sieved using a 100 mesh (150 μm) screen to remove the particles too large for the Bahco Classifier to handle. No effort was made to grind the dye particles which did not pass through the 100 mesh screen, except for applying gentle pressure with a small spatula to spherical agglomerates. The model 6000 Bahco was calibrated and the particle size analysis was done according to ASME (American Society of Mechanical Engineers) Powder Test Code 28. The results for each of the dye mixes are summarized in Table 4. The particles less than 10 μm in diameter are considered respirable. The particles greater than 150 μm are those which were too large to be handled by the Bahco.

TABLE 4. PARTICLE SIZE ANALYSIS OF DYE POWDERS, AS RECEIVED

	Percent Particles (Weight)		
Dye Mix	< 10 μm (a)*	> 150 μm (g)**	d_a***
Violet	29	9.1	20
Green	22	8.1	31
Yellow	40	6.4	14
Red	25	40	32

*a = aerodynamic diameter
**g = geometric diameter
***d_a = aerodynamic median diameter

RESULTS AND DISCUSSION

In this section, the results of the separation and identification of the smoke dye components are presented. Additionally, the Edgewood dye standards DBC and BZA have been separated into their components and analyzed, and these results are also reported. Both of these dye standards were found to contain an appreciable amount of impurities. These compounds are major components of both the YSM and GSM and therefore it was desirable to devise separation schemes for them prior to the study of the more complex smoke mixes. The other three dye standards, MAA, PTA, and DDA, were found to be greater than 98 percent pure and thus are not included in this section.

Dye Standards

Benzanthrone (BZA). The BZA received was stated to be nearly 99 percent pure. However, a substantial amount of chloroform insoluble (19.7 percent) and/or nonvolatile (19.3 percent at 245°C and 75 mtorr)

material was found during the separation of this material. Studies showed that these two materials were essentially identical. The elemental analysis of the two materials is given below:

	C	H	O	N	Total (%)
BZA Insolubles	43.2	4.0	39.4	0.2	86.8
BZA Non-Volatiles	48.5	3.5	35.2	0.1	87.4

There appear to be other elements present in the sample which were not included in the elemental total of 87 percent. The infrared spectra of these two materials were also similar. The infrared spectrum of the sublimation residue appeared to be quite complex with a substantial amount of hydroxyl content.

The separation of BZA is outlined in Figure 2. It was later incorporated into the separation scheme of the YSM. By TLC, all three volatile fractions appeared to be nearly pure BZA with only traces of impurities in fractions 1 and 3. The GC/MS analyses of these three volatile fractions showed the presence of anthraquinone; however, the amount present decreased from fraction 1 to fraction 3. Fraction 2, however, was thought to be the most pure sample of BZA because fraction 3 contained a small amount of the black sublimation residue. An infrared spectrum of the chloroform soluble portion of BZA is shown in Figure 8.

The ^{13}C NMR spectra of the chloroform soluble portion of the BZA standard is shown in Figure 9. This spectrum corresponds to the spectrum expected for benzanthrone, except for a few small impurity peaks that correspond to those observed in the ^{13}C spectrum of anthraquinone. The presence of anthraquinone in this material was verified by GC/MS. A purified BZA sample, fraction 2, was used to obtain the ^{1}H NMR spectrum in Figure 10. This spectrum, except for the solvent peak and a solvent impurity (both indicated) is consistent with that of benzanthrone.

Dibenzochrysenedione (DBC). The DBC standard received was stated to be about 80 percent pure. The separation scheme for DBC is shown in Figure 3. This scheme was later incorporated into the separation procedure for the YSM. Fraction 1 was collected up to 245°C at 75 mtorr. The TLC of this fraction indicated the presence of at least six compounds, including BZA and DBC. Direct probe mass spectral analysis indicated the presence of BZA, DBC, two diketones with molecular weights of 336 and 366, a phenyl-substituted diketone with a molecular weight of 334 (possibly phenylchrysenedione) and several other compounds which could not be unambiguously identified. Fraction 2 was found by TLC to contain fewer impurities than fraction 1. The direct probe mass spectra of fraction 2 revealed the presence of a number of compounds, including DBC, and the same 336 and 366 molecular weight compounds found in fraction 1.

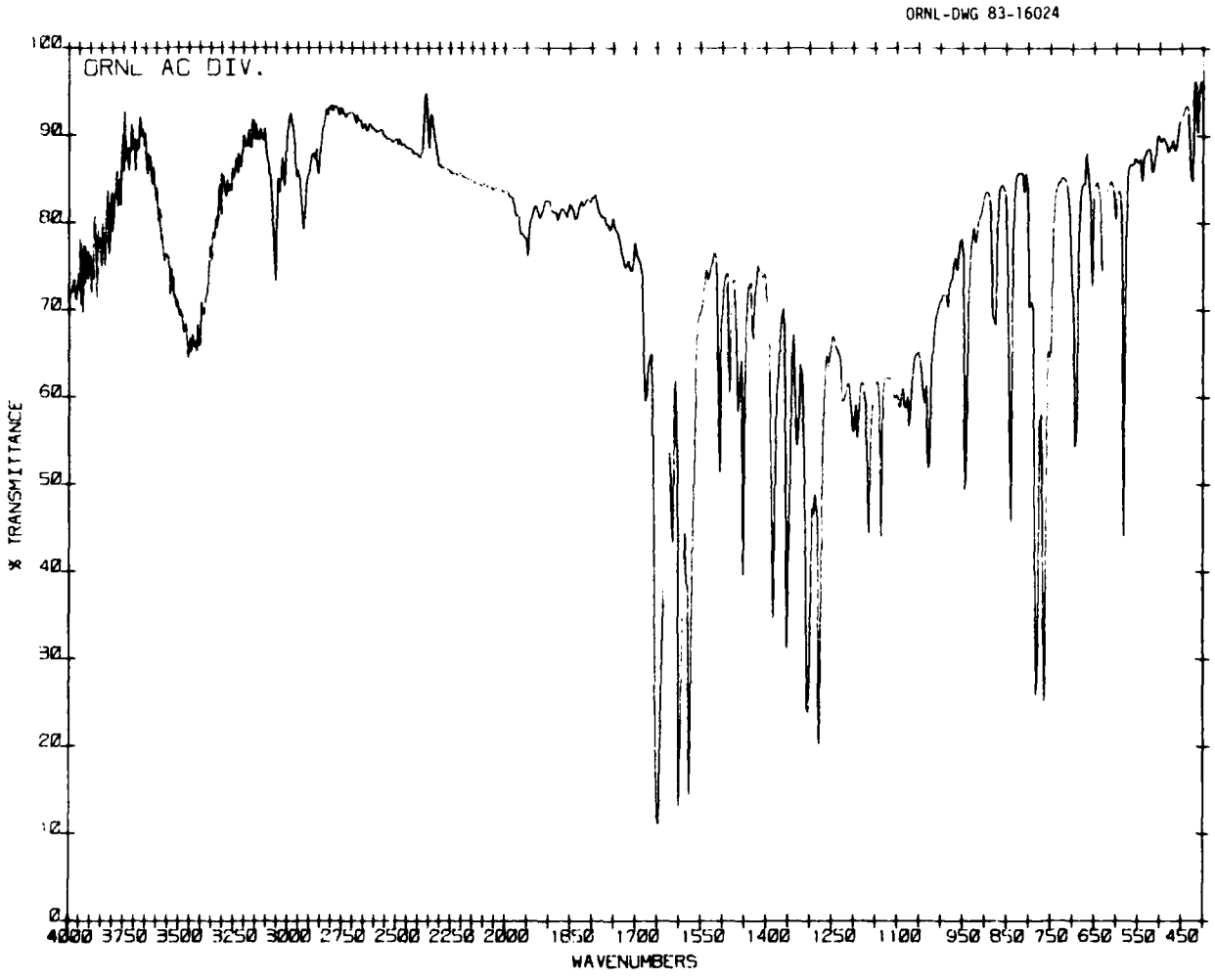

Figure 8. Infrared Spectrum of Benzanthrone (BZA)

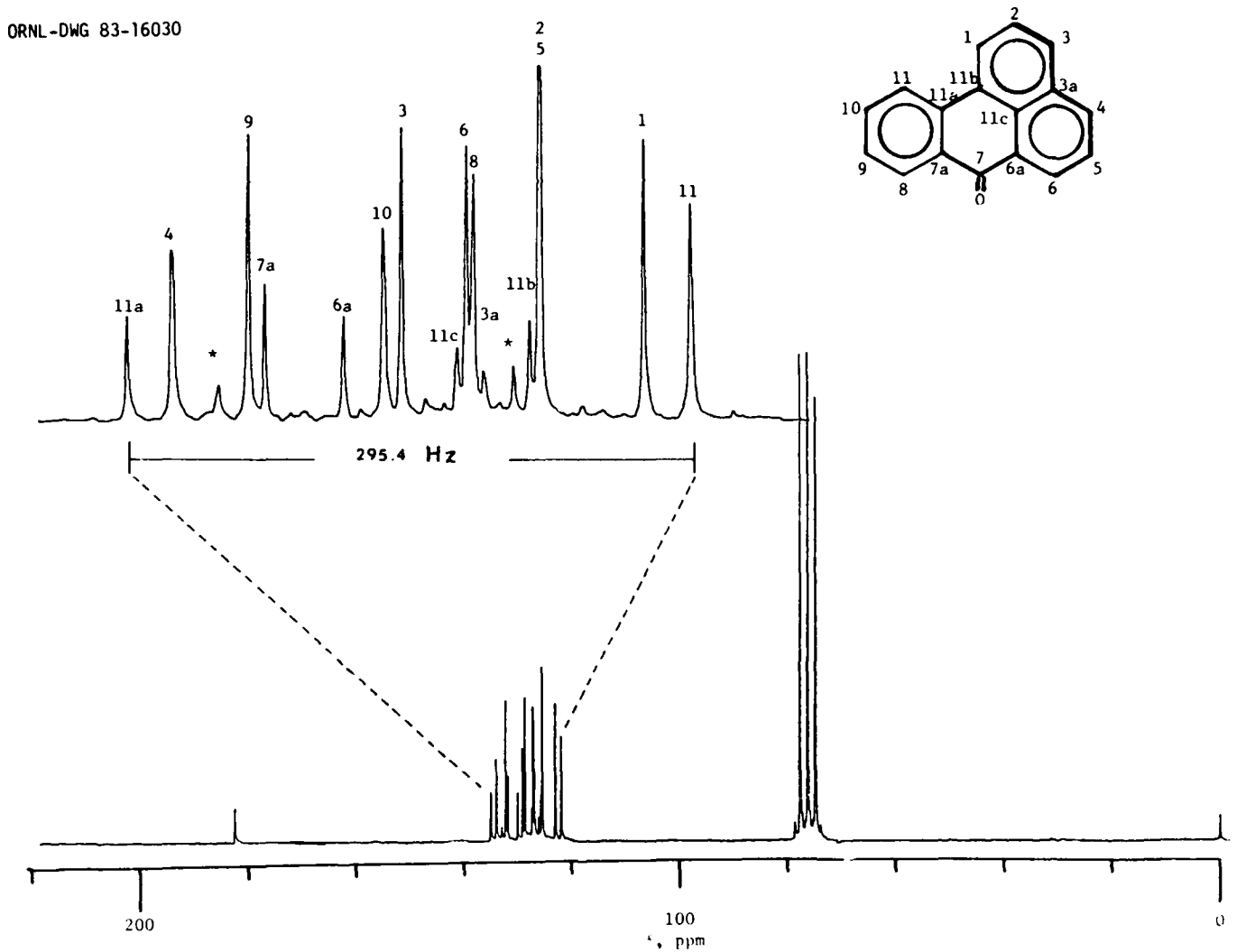

Figure 9. Carbon-13 NMR Spectrum of the Chloroform-Soluble Portion of Benzanthrone (BZA) in $CDCl_3$. *Starred peaks (*) are due to the presence of anthraquinone.

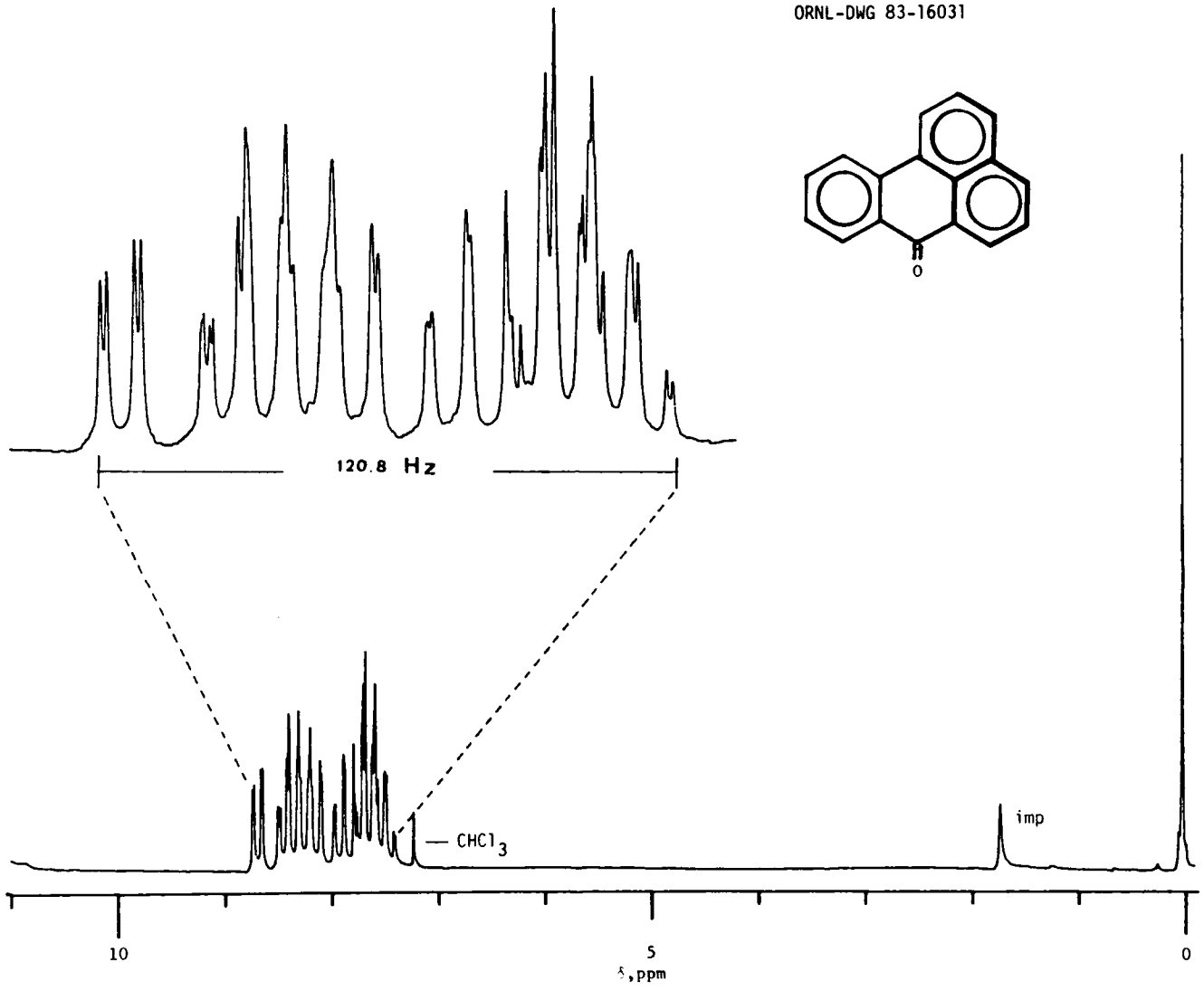

Figure 10. Proton NMR Spectrum of Benzanthrone (BZA) Dye Standards, Fraction 2, in $CDCl_3$

The third fraction contained one major band, with a smaller impurity. The major band corresponded to DBC. When it was analyzed by direct probe mass spectrometry, both DBC and dibenzochrysene were identified in this fraction. The residue was found to contain DBC, the 366 diketone, and a large amount of high molecular weight material, which was not volatilized. The infrared, ^{1}H NMR, and ^{13}C NMR spectra of DBC fraction 3, are given in Figures 11-13, rspectively. The ^{13}C NMR spectra of this fraction suggested that this fraction was essentially pure DBC. The resulting line assignments are given in Figure 13. The low solubility of DBC in organic solvents required the solution ^{13}C NMR spectrum of DBC to be obtained in concentrated sulfuric acid. For comparison, the solid ^{13}C NMR spectrum of DBC was also obtained, courtesy of M. Albright of JEOL (USA), Inc. This solid state spectrum is shown in Figure 14.

Colored Smoke Mixes

Red Smoke Mix (RSM). The RSM is formulated to contain only one dye component, 1-methylaminoanthraquinone (MAA).[4] The infrared, ^{1}H and ^{13}C NMR spectra of MAA are shown in Figures 15-17 for reference. The spectral lines of the ^{13}C NMR spectrum for MAA were assigned using proton noise decoupling in conjunction with undecoupled spectra with nuclear Overhauser enhancement, and spin-lattice relaxation time (T_1) measurements. In addition to MAA, the RSM was also found to contain over 10 percent of a non-volatile, chloroform insoluble material as well as much smaller quantities of other impurities. Analytical scale high performance liquid chromatography (HPLC) studies of RSM showed five or six components present. Because a preparative scale HPLC was not available, the RSM was separated into three fractions using vacuum sublimation (see Figure 4).

Analysis of the first vacuum sublimation fraction (up to 120°C at 75 mtorr) by TLC indicated that it was composed primarily of MAA with at least five impurities. Gas chromatography showed the presence of nearly 25 impurities, although most of these were present in trace amounts. Combined GC/MS identified the largest impurity in fraction 1 as anthraquinone (about 25-30 percent of the MAA by area). Other peaks identified at the 1 percent or lower level include compounds with molecular weights of 223 (aminoanthraquinone), 182 (azobenzene), 198 (azoxybenzene), 169 (aminobiphenyl), 258 (phenyldiazobenzene) and 284 (a diketone of a condensed ring compound with a molecular weight of 254, such as dibenzoacenaphthylene or phenylanthracene).

Fraction 2 (74 percent, 120-190°C) was found to contain primarily MAA, with a small amount of anthraquinone and trace amounts of several other impurities. The UV/VIS and fluorescence spectra of fraction 2 were identical to the MAA dye standard spectra. The infrared, ^{1}H and ^{13}C NMR spectra of fraction 2 and the MAA dye standard are also identical.

Fraction 3 was a black powder which was non-volatile at 190°C and 75 mtorr. When it was placed in chloroform, about 13 percent of it was

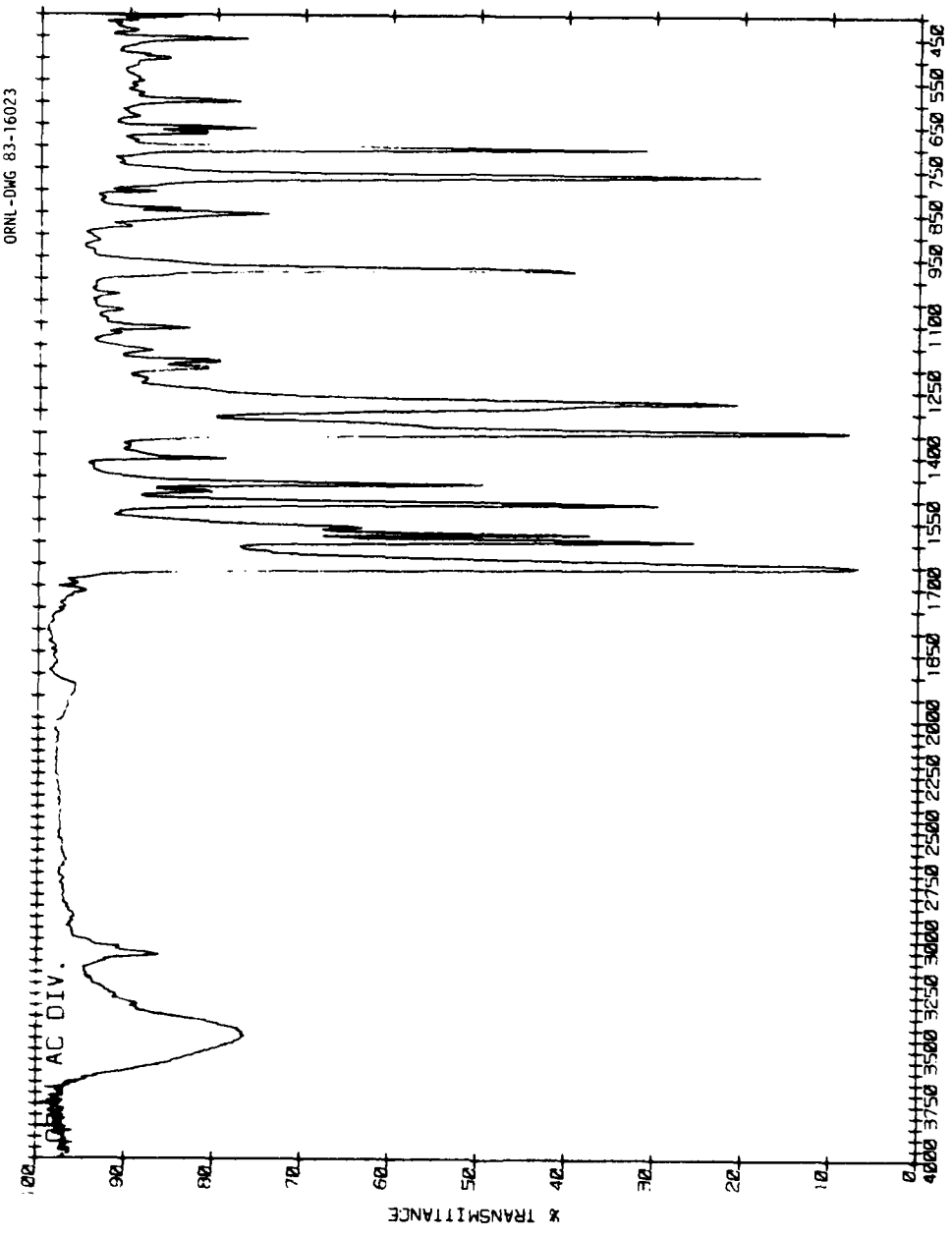

Figure 11. Infrared Spectrum of Dibenzochrysenedione (DBC), Fraction 3

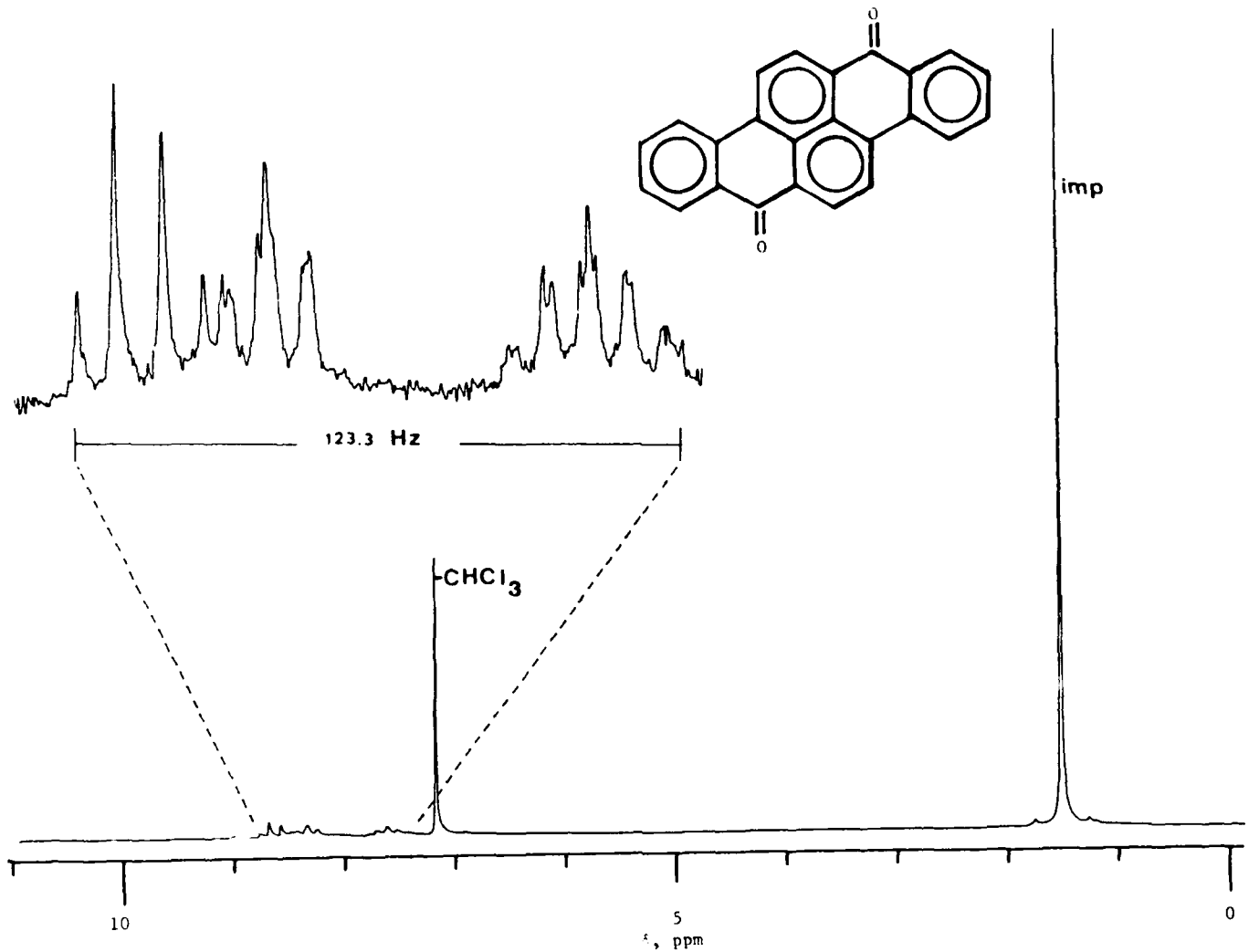

Figure 12. Proton NMR Spectrum of Dibenzochrysenedione (DBC) Dye Standard, Fraction 3, in $CDCl_3$

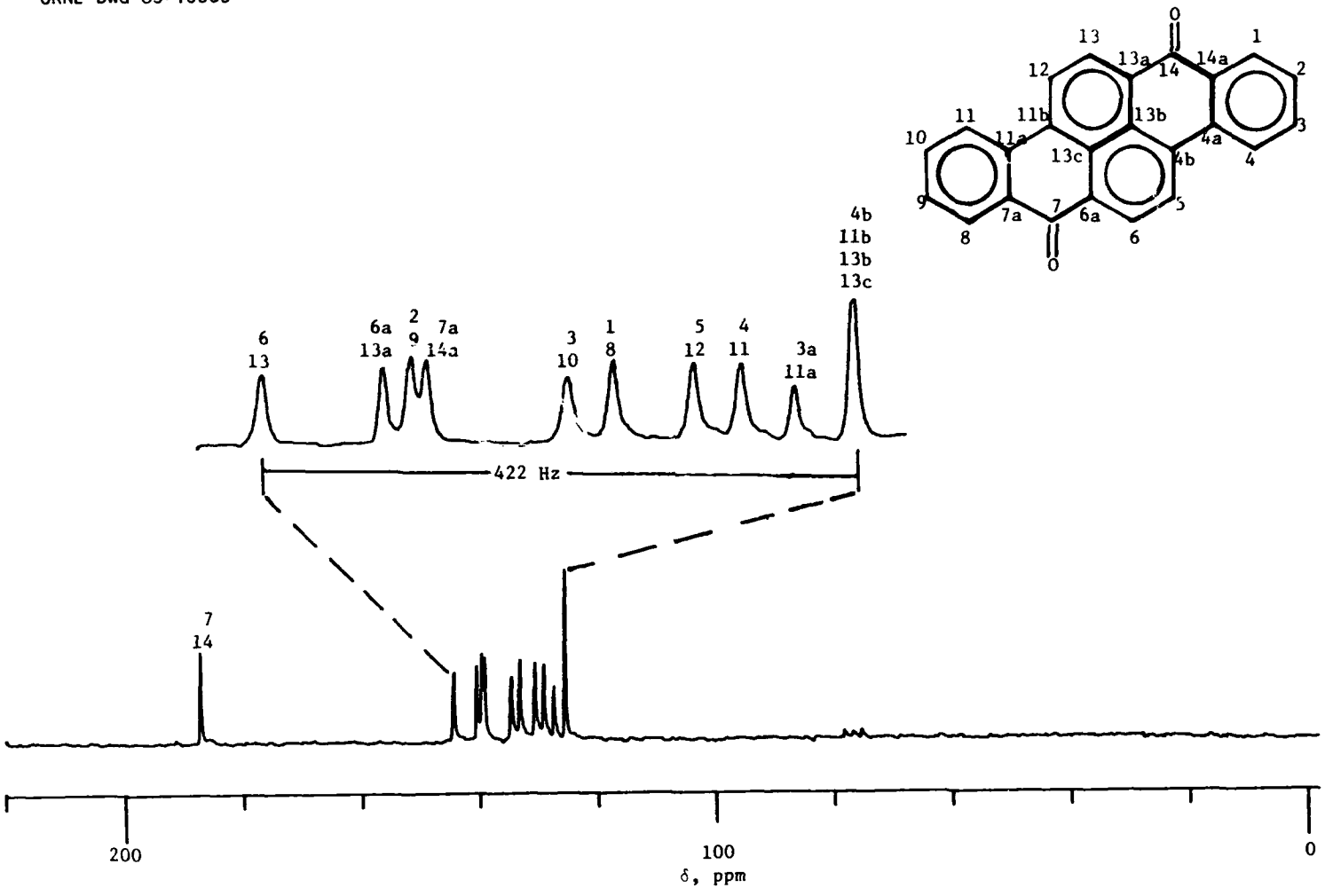

Figure 13. Carbon-13 NMR Spectrum of Dibenzochrysenedione (DBC) in H_2SO_4

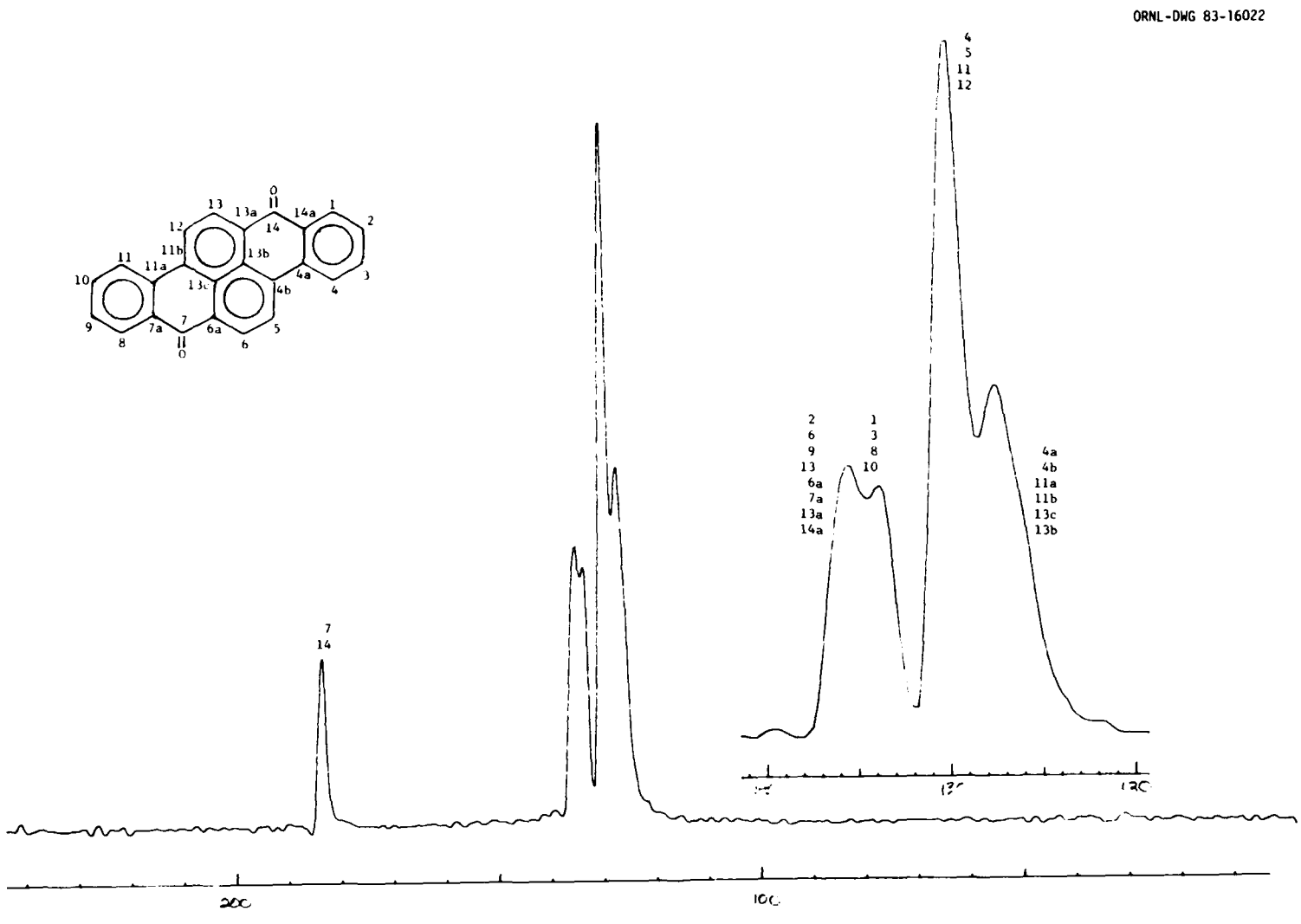

Figure 14. Solid Probe Carbon-13 NMR Spectrum of Dibenzochrysenedione (DBC) Dye Standard, Fraction 3

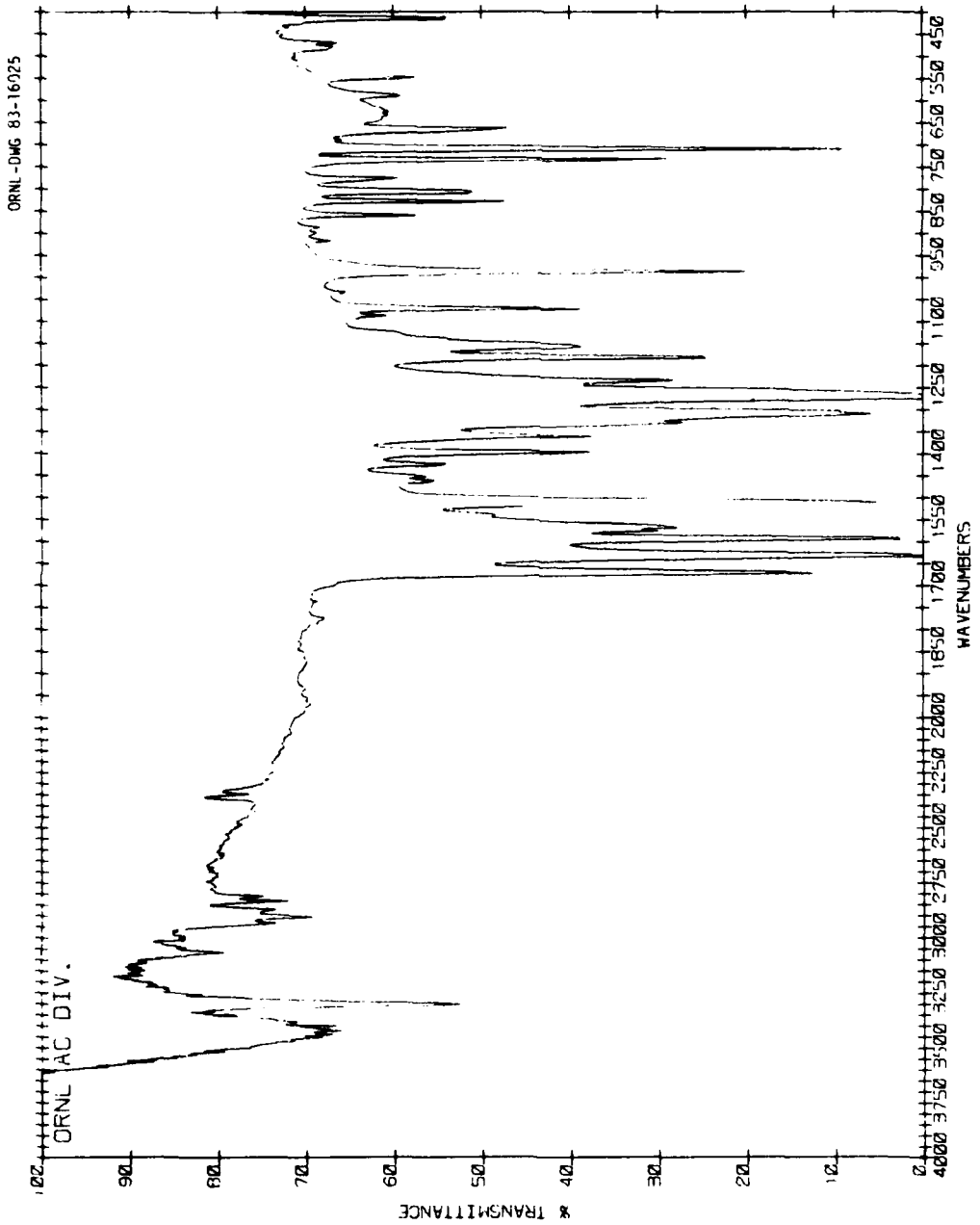

Figure 15. Infrared Spectrum of 1-Methylaminoanthraquinone (MAA)

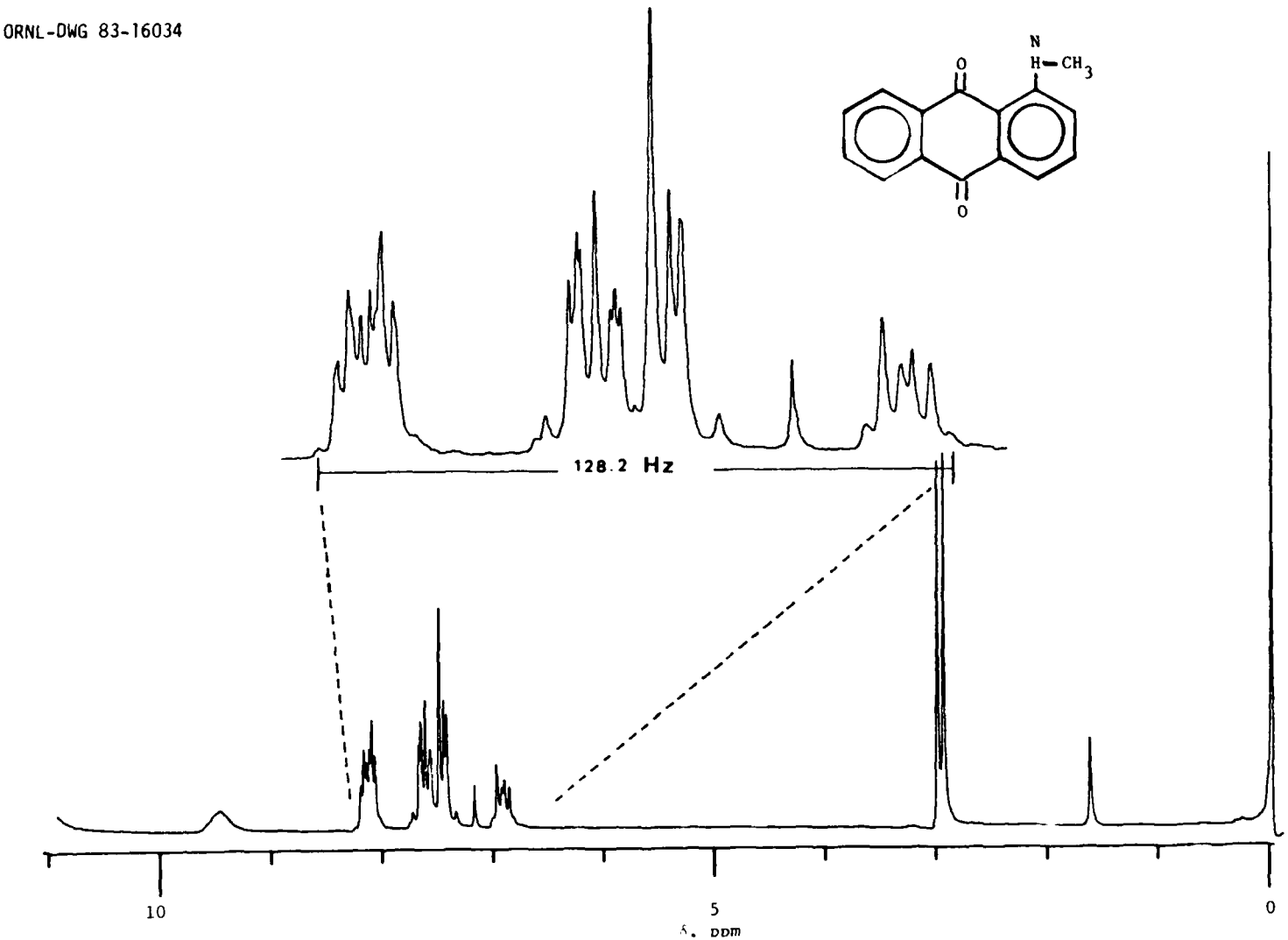

Figure 16. Proton NMR Spectrum of 1-Methylaminoanthraquinone (MAA) in $CDCl_3$ Dye Standard

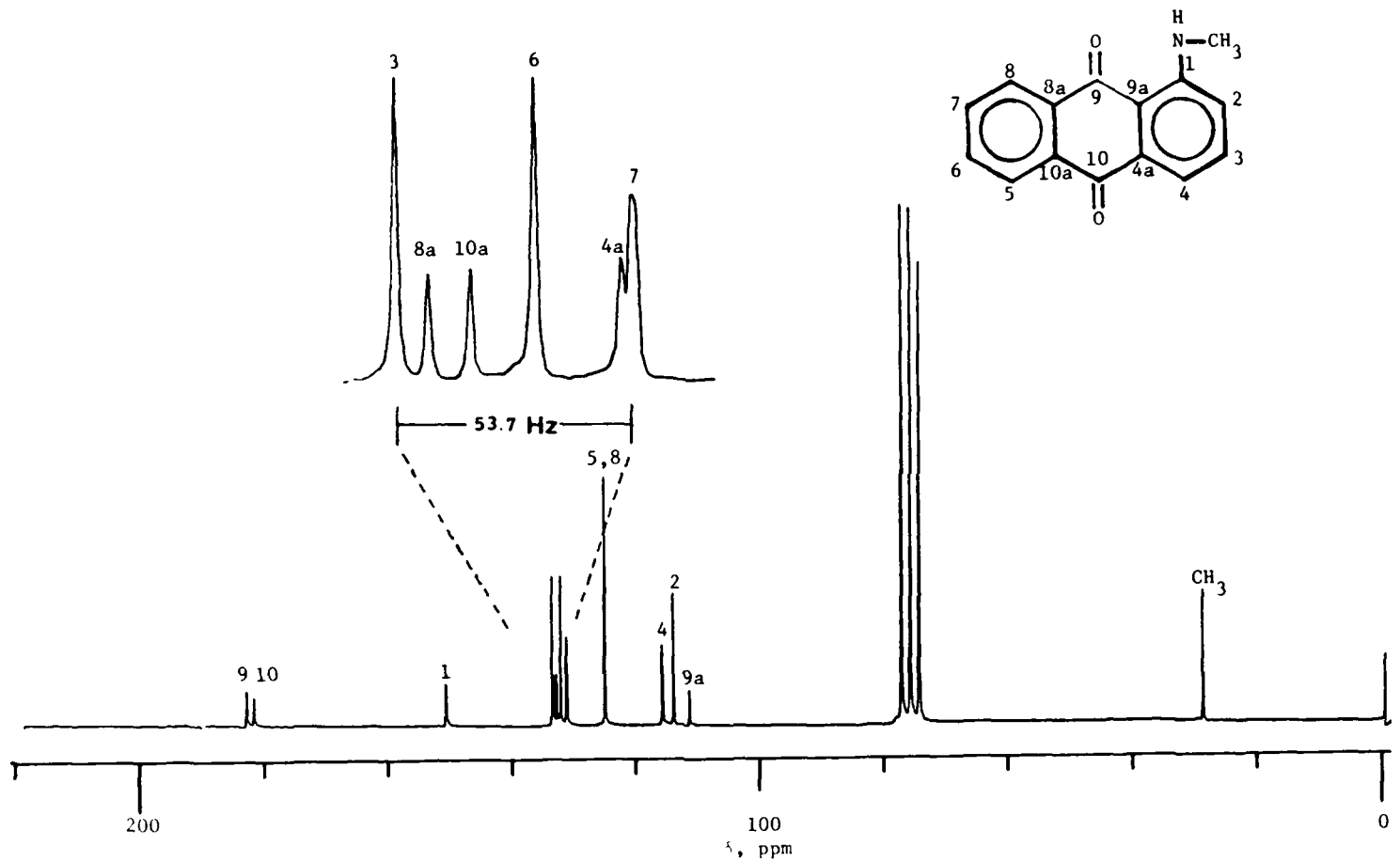

Figure 17. Carbon-13 NMR Spectrum of 1-Methylaminoanthraquinone (MAA) in $CDCl_3$ Dye Standard

soluble, producing a red color, which was due to residual MAA. To test if the sublimation residue was present in the original RSM or if it was a thermal degradation product, the RSM was dissolved in chloroform and the insoluble material was filtered, dried, and weighed. The insoluble material (not passing through a 5 μm filter) was found to represent 13.3 percent of the original RSM. This non-soluble material and the sublimation residue were tested by elemental analysis and IR spectroscopy. The results of the elemental analysis are given below:

	C	H	N	O	Total (%)
RSM Insolubles	40.6	6.0	0.49	49.8	96.9
RSM Residue	41.3	5.9	0.68	46.5	94.4

The infrared spectrum of fraction 3 is shown in Figure 18. The RSM insolubles yielded a similar spectrum. These data confirm the idea that the vacuum sublimation residue is not a thermal decomposition product, but is a component of the original smoke mix. The elemental analysis yields an empirical formula of CH_2O, assuming the small amount of nitrogen detected is from residual MAA. The infrared spectrum (Figure 18) indicates that the residue contains oxygen present as hydrogen-bonded hydroxyl groups.

Violet Smoke Mix (VSM). The VSM is formulatd to contain 80 percent 1,4-diaminodihydroanthraquinone (DDA) and 20 percent 1-methylaminoanthraquinone (MAA).[4] In the preliminary studies of the VSM, the analyses of DDA were complicated by changes in the amounts of DDA detected. The TLC of DDA solutions consistently resulted in two spots, one yellow-brown and the other purple, with a large R_f value difference. Packed column GC (on Dexsil 400) showed only one peak present in the analysis of the DDA standard, but capillary column GC/MS (on OV-101) showed two peaks with molecular weights of 238 and 240, corresponding to DDA and diaminoanthraquinone (DAA), respectively.

When DDA was analyzed by ^{13}C NMR (in DMSO-d_6), a spectrum consistent with DDA was obtained (see Figure 19). However, when this solution was allowed to heat up in the NMR probe overnight the solution color changed from yellow-brown to purple. The ^{13}C NMR spectrum of the purple solution indicated that it was DAA (Figure 20), with the saturated carbon at 27.5 ppm in the DDA spectrum shifting to the aromatic region. Proton spectra of DDA and DAA in $CDCl_3$ are shown in Figures 21 and 22.

The conversion of DDA could be observed by a TLC separation of DDA. Approximately 25 mg of DDA was streaked on a silica preparative scale plate and developed with a 80:60:1 solution of methylethylketone, chloroform and acetic acid. A purple band was readily separated prior to the second yellow-brown band. The purple band was removed, analyzed by capillary column GC/MS (OV-101) and visible spectrophotometry, and identified as almost pure DAA. The second band could be observed to change to a purple color as it was developed on the TLC plate. When

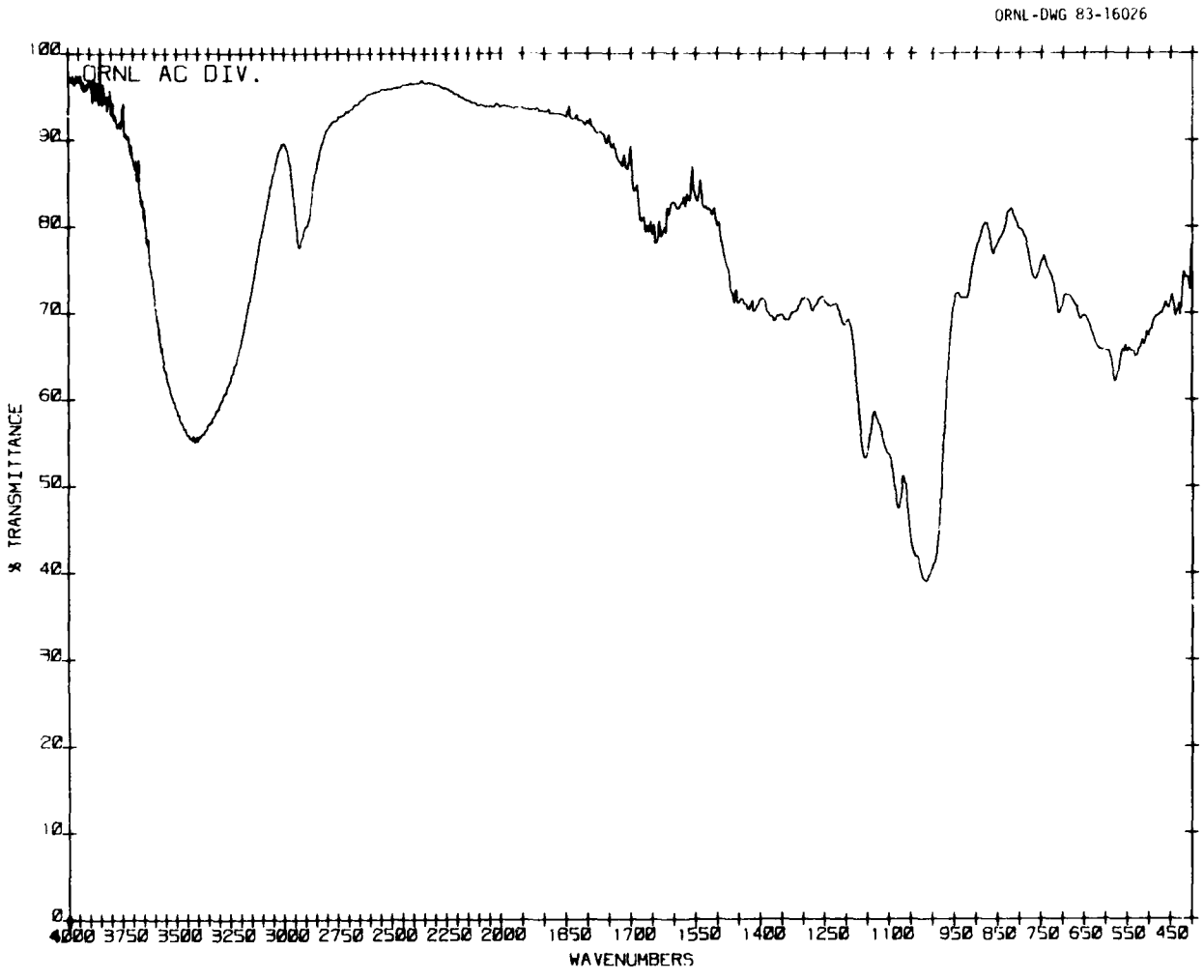

Figure 18. Infrared Spectrum of Red Smoke Mix Non-Volatile Residue (Fraction 3)

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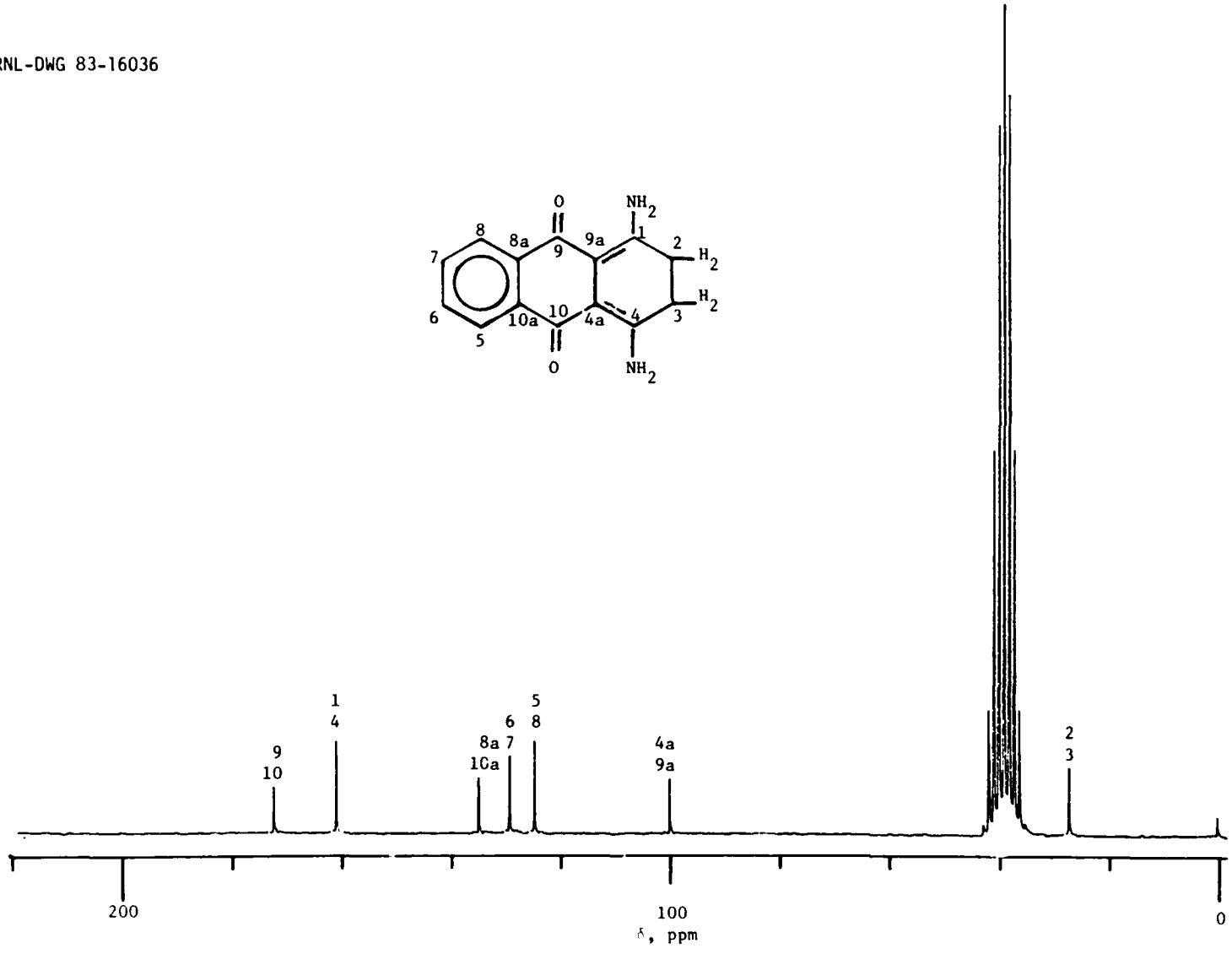

Figure 19. Carbon-13 NMR Spectrum of 1,4-diamino-2,3-dihydroanthraquinone (DDA) in DMSO-d_6

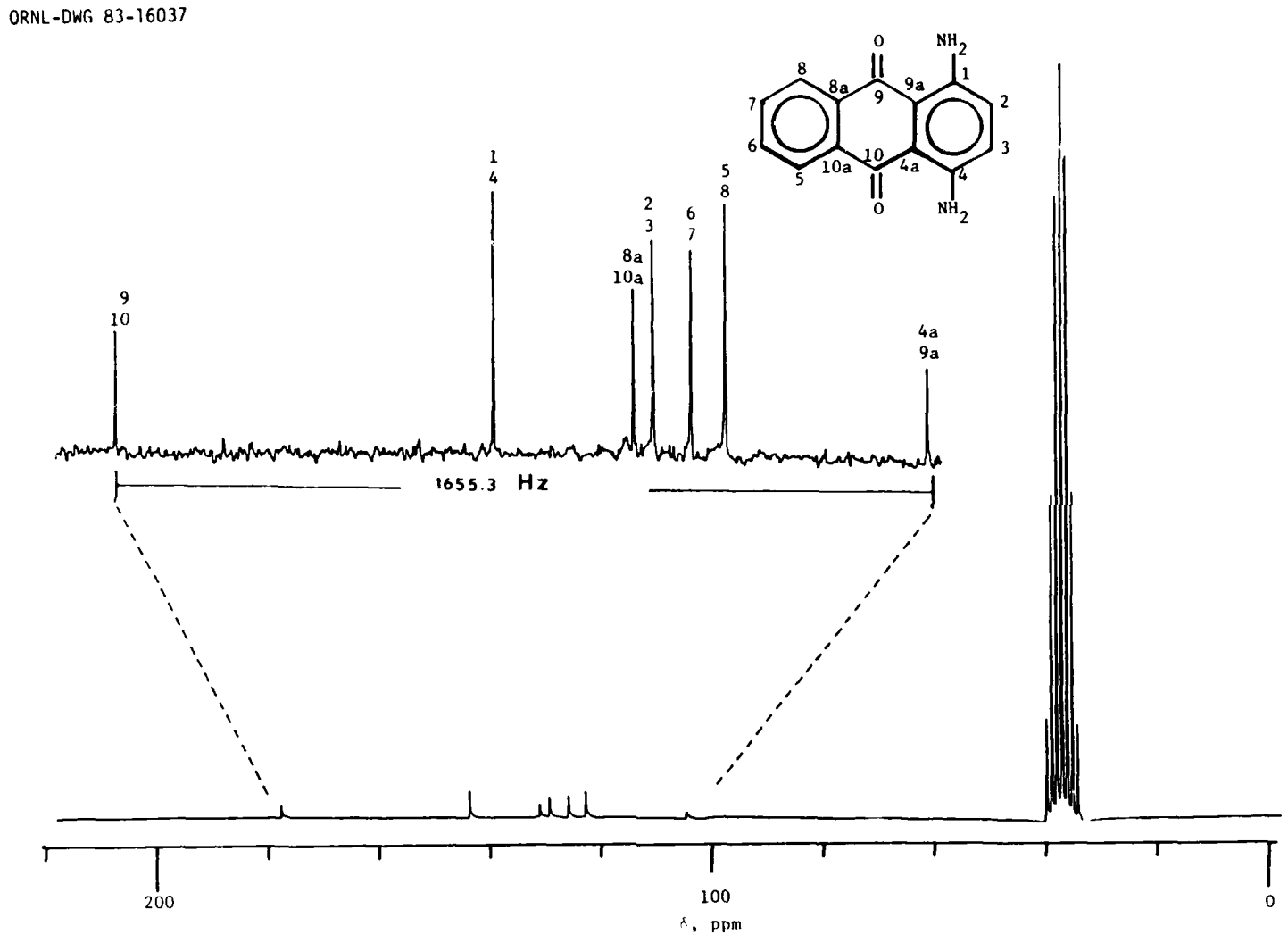

Figure 20. Carbon-13 NMR Spectrum of 1,4-diaminoanthraquinone (DAA) in DMSO-d_6

ORNL-DWG 83-16038

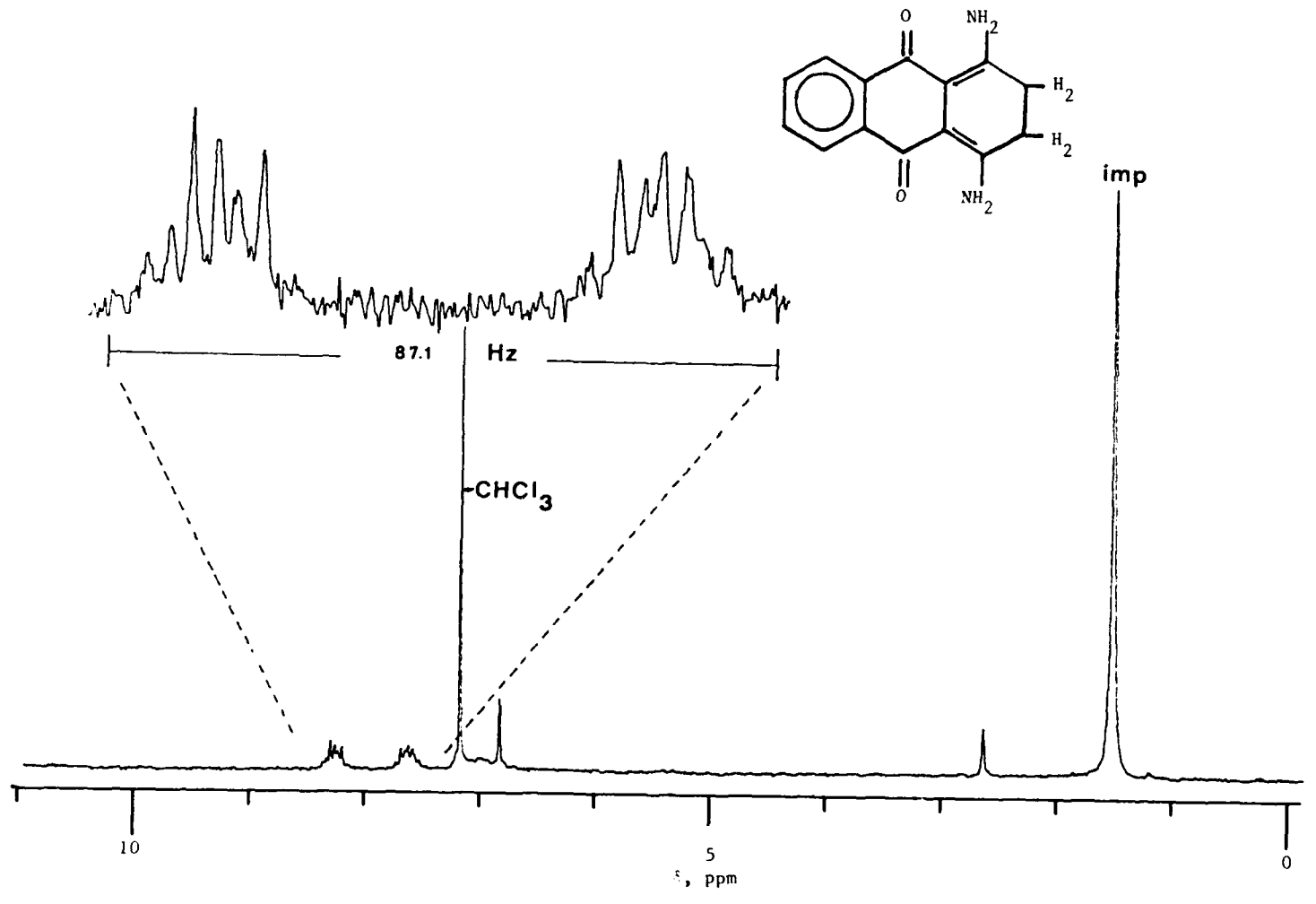

Figure 21. Proton NMR Spectrum of 1,4-diamino-2,3-dihydroanthraquinone (DDA) in $CDCl_3$

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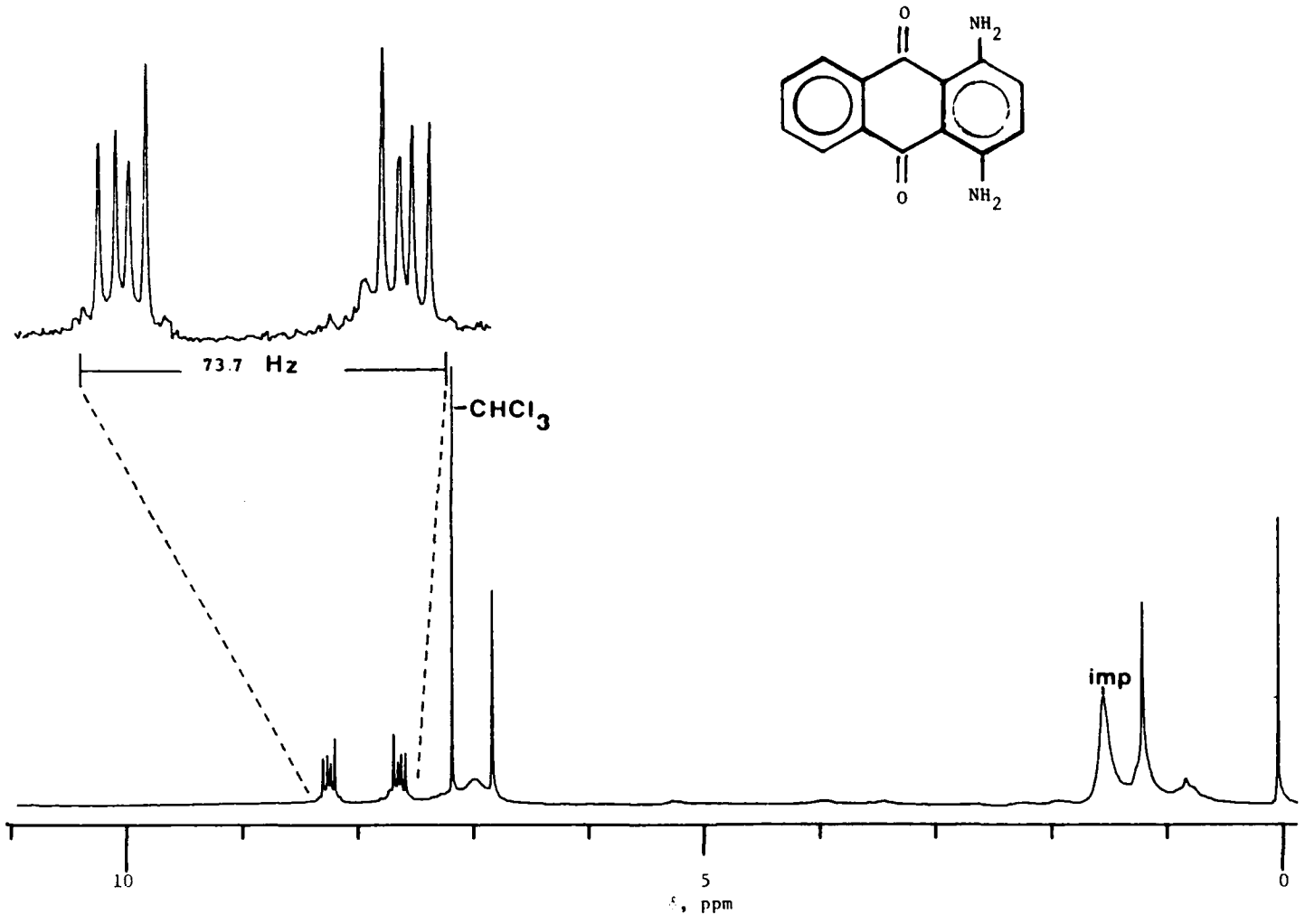

Figure 22. Proton NMR Spectrum of 1,4-diaminoanthraquinone (DAA) in $CDCl_3$

the plate was dried, however, the color changed to brown. Analysis of this band showed that it was a mixture of DDA and DAA. The visible spectra of DDA and DAA are shown in Figure 23. The spectra are similar in shape and intensity but differ by about 100 nm in wavelength. The oxidation of DDA to DAA also explains the spectral shift described by Owens and Ward[4] in their study of the visible spectra of VSM before and after ignition. Infrared spectra of DDA and DAA are shown in Figures 24 and 25.

The separation of VSM by differential solubilities as outlined in Figure 5 is based upon the fact that MAA is nearly 70 times more soluble in chloroform than is DDA. The TLC of fraction 1 indicated that the major component is MAA, but there was also a purple streak of a lower R_f value. (This purple compound was later identified as diaminoanthraquinone, DAA). Analysis by GC/MS showed that the major component of fraction 1 was MAA, with several components present at less than 1 percent. The minor impurities identified include anthraquinone (molecular weight of 208), DDA (240), aminonaphthalene (143), aminoanthraquinone (223), phenyldiazobenzene (258), and DAA (238).

Fraction 2, which represents only 3.6 percent of VSM, was found to contain mostly DDA, with varying amounts of DAA (depending upon sample treatment as explained previously), small amounts (approximately 1 percent) of MAA (257), aminoanthraquinone (223), aminonaphthalene (143), and anthraquinone (208).

Fraction 3 (52.6 percent), when examined by TLC and visible, UV, and fluorescence spectrophotometry, was found to contain both DDA and DAA. A visible spectrum of this fraction containing both compounds is shown in Figure 23. Combined glass capillary GC/MS showed aminonaphthalene (present at less than 1 percent) as the only impurity. The amounts of DDA and DAA present in the GC/MS of this fraction were also found to vary with sample treatment.

The insoluble residue, fraction 4, is a gray powder that represents 24 percent of the original sample. The direct probe mass spectra obtained on this sample were too complex to allow the identification of specific compounds; however, a very small amount of DAA was detected.

Yellow Smoke Mix (YSM). The YSM is formulated to contain benzanthrone (BZA) and dibenzochrysenedione (DBC) in a ratio of 64:36.[4] The BZA is substantially more soluble and more volatile than DBC, so differential solubility or vacuum sublimation would seem to be viable methods to separate this mixture. However, it was quickly discovered that a relatively soluble compound was present as an impurity in the DBC, making differential solubility less useful. Therefore, vacuum sublimation was used to separate the YSM into four volatile fractions and one non-volatile fraction. This last fraction was separated further, as outlined in Figure 6.

Both fractions 1 ($T < 130°C$, 75 mtorr, 5.7 percent) and 2 (130-175°C, 28.6 percent) appeared to contain only BZA when analyzed by TLC.

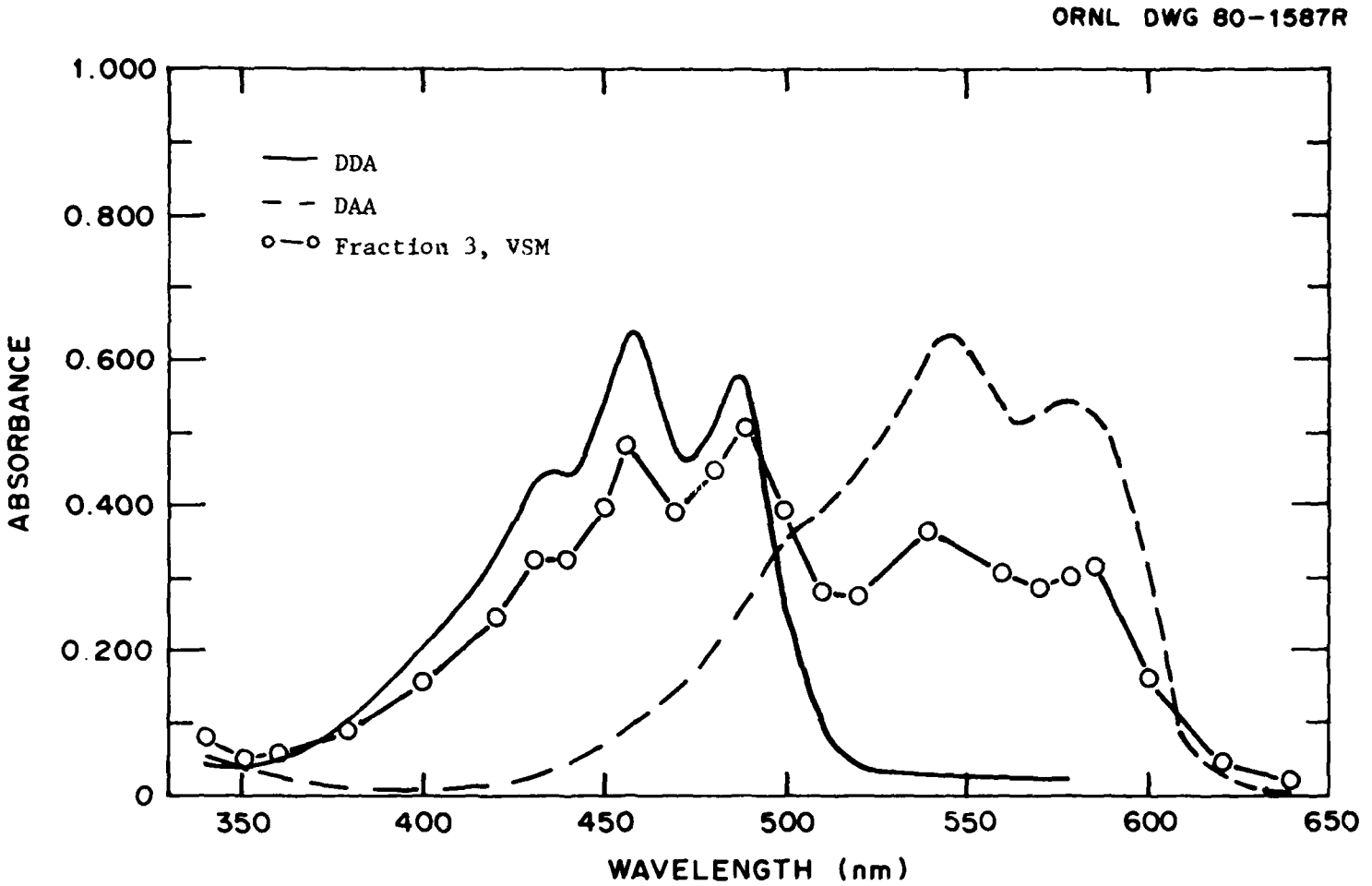

Figure 23. Visible Spectra of 1,4-diamino-2,3-dihydroanthraquinone (DDA), 1,4-diaminoanthraquinone (DAA) and Fraction 3 of Violet Smoke Mix

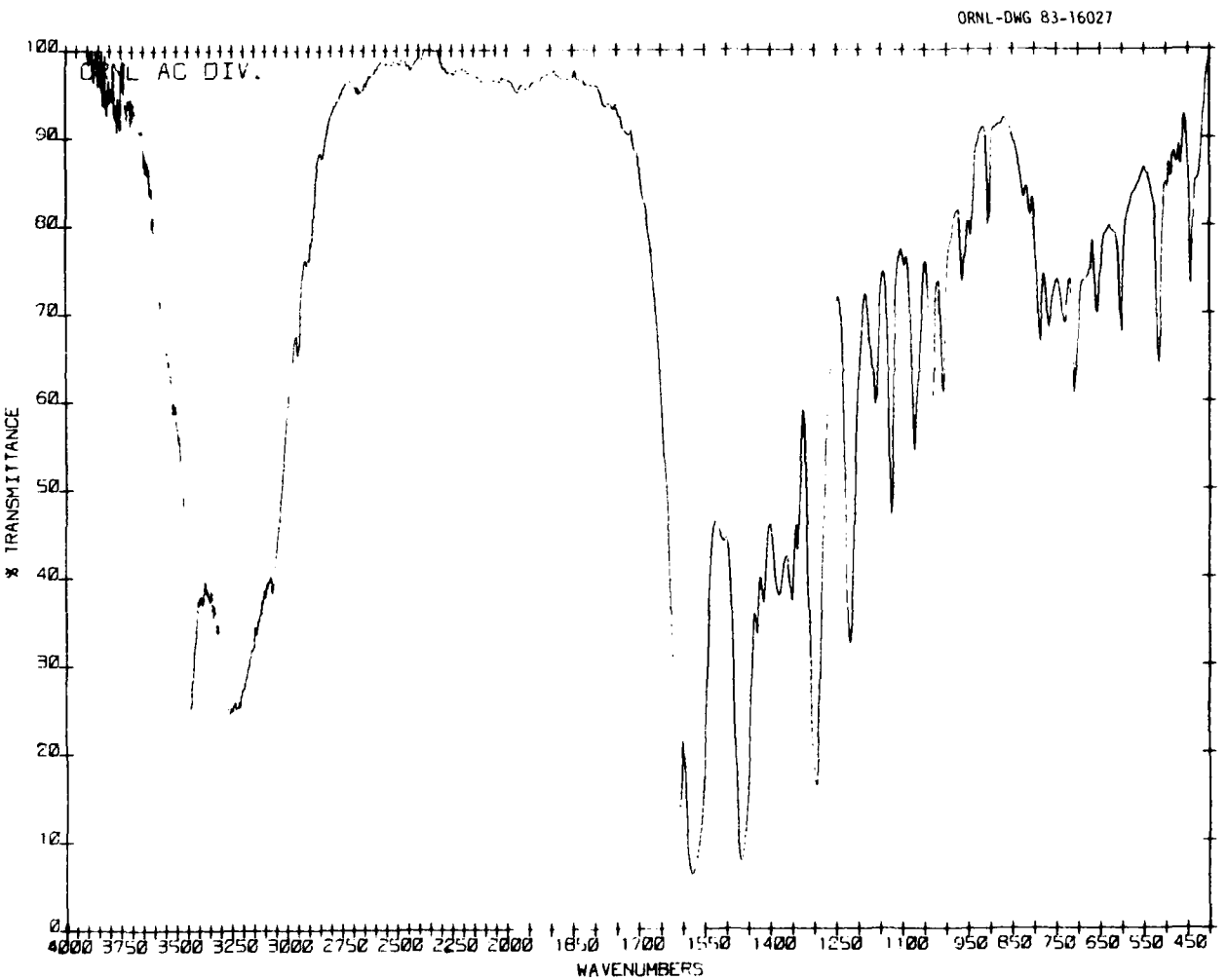

Figure 24. Infrared Spectrum of 1,4-diamino-2,3-dihydroanthraquinone (DDA)

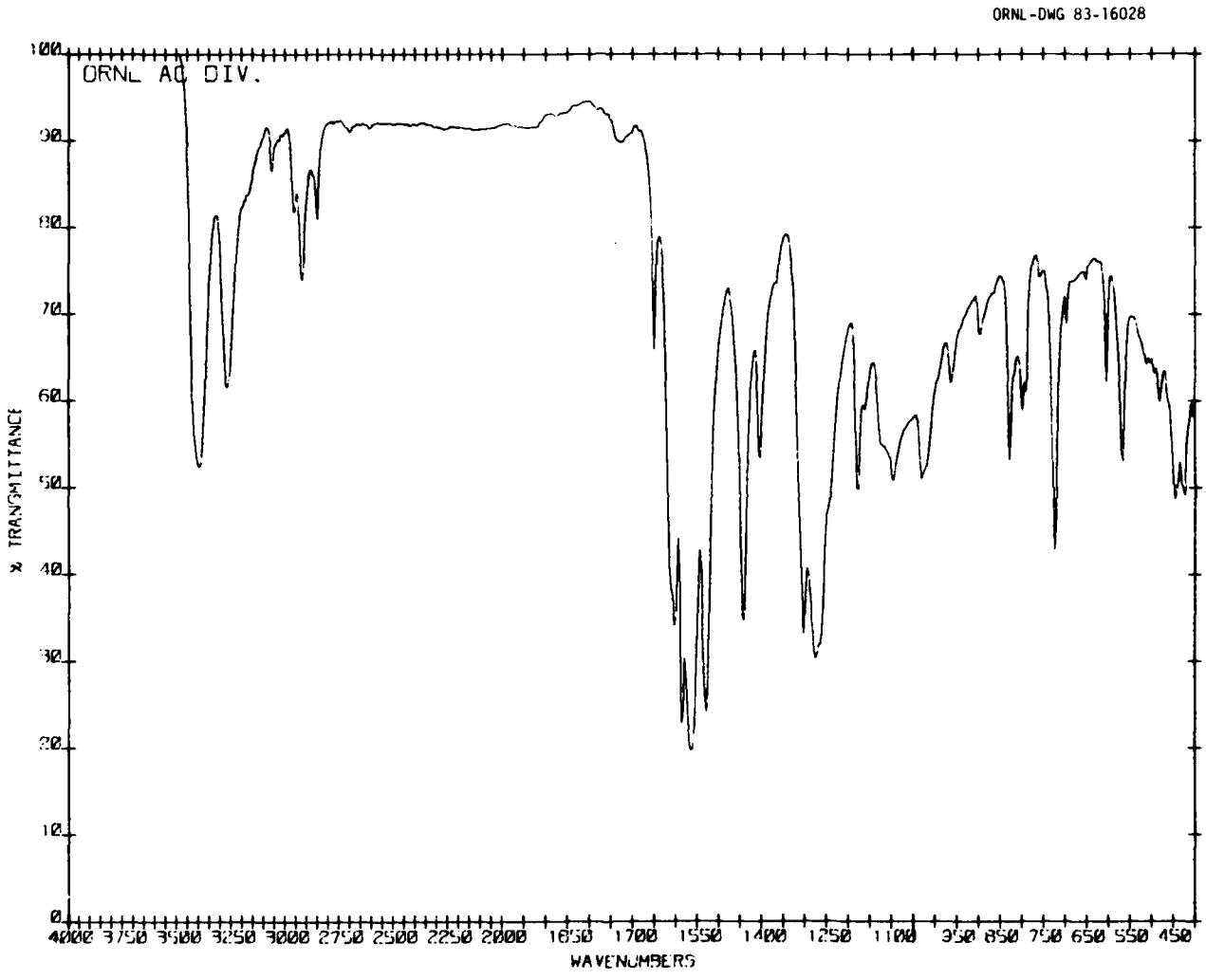

Figure 25. Infrared Spectrum of 1,4-diaminoanthraquinone (DAA)

Capillary column GC/MS (on SE-52) revealed the presence of a number of hydrocarbon impurities in fraction 1, however. These hydrocarbons are thought to be from the antidusting agent added to the DBC. This antidusting reagent is reported to be Marcol 52.[4] A sample of this, obtained from Exxon, was found by GC/MS to contain a number of long chain hydrocarbons, similar to those observed in fraction 1. Fraction 2 was also found to contain a small impurity (< 1 percent), which was identified as anthraquinone by GC/MS. The visible spectrum of fraction 2 was found to be identical to the BZA standard.

Fractions 3 and 4, when analyzed by GC/MS, were found to contain only BZA. However, TLC revealed several other small impurities which were probably non-volatile and thus not gas chromatographable. For example, the direct probe MS of fraction 4 revealed the presence of some DBC in addition to the BZA detected by GC/MS. Fraction 5, which was brown, was found using direct probe MS to contain BZA, DBC and an unidentified compound (a diketone with a molecular weight of 366). These spectra were too complex, however, to identify minor components. Using GC/MS, BZA was the only compound volatile enough to be eluted in fraction 5.

Fraction 6 was found to contain mostly DBC by direct probe MS, plus the impurity with a molecular weight of 366, which was also observed in fraction 5. Only two compounds were observed by TLC in this fraction. The visible and ultraviolet spectra of fraction 6 were found to be quite similar to those of pure DBC. The direct probe mass spectra of fraction 7 were very complex, with only DBC being clearly identified. Also, after the direct probe analysis was completed, a substantial amount of non-volatilized material remained in the sample cup, indicating that this fraction contains a substantial amount of high molecular weight material. The infrared spectrum of this fraction was very complex, but exhibited substantial hydroxyl content.

Green Smoke Mix. The GSM is formulated to contain 1,4-di-p-toluidino-9,10-anthraquinone (PTA), benzanthrone (BZA) and dibenzochrysenedione (DBC) in a ratio of 70:20:10.[4] The infrared and ^{1}H and ^{13}C NMR spectra of PTA are given in Figures 26-28, respectively, for reference. The ^{13}C NMR line assignments are based on proton noise decoupled spectra in conjunction with undecoupled spectra with nuclear Overhauser enhancement, and spin-lattice relaxation-time (T_1) measurements. The BZA and PTA of the GSM can be readily separated from DBC by either vacuum sublimation or differential solubility. However, BZA and PTA sublime throughout a wide temperature range (up to 245°C at 75 mtorr) and thus cannot be separated using vacuum sublimation. The two compounds have similar solubilities in a wide range of solvents and thus do not allow separation on the basis of solubility. Although BZA, PTA, and DBC can be resolved on analytical scale silica TLC plates, the separation of whole GSM on preparative scale TLC plates was very poor. Column chromatography using alumina had been shown to be successful in separating some anthraquinone derivatives,[1,5] so this approach was tried and finally used in the separation of the GSM, as outlined in Figure 7.

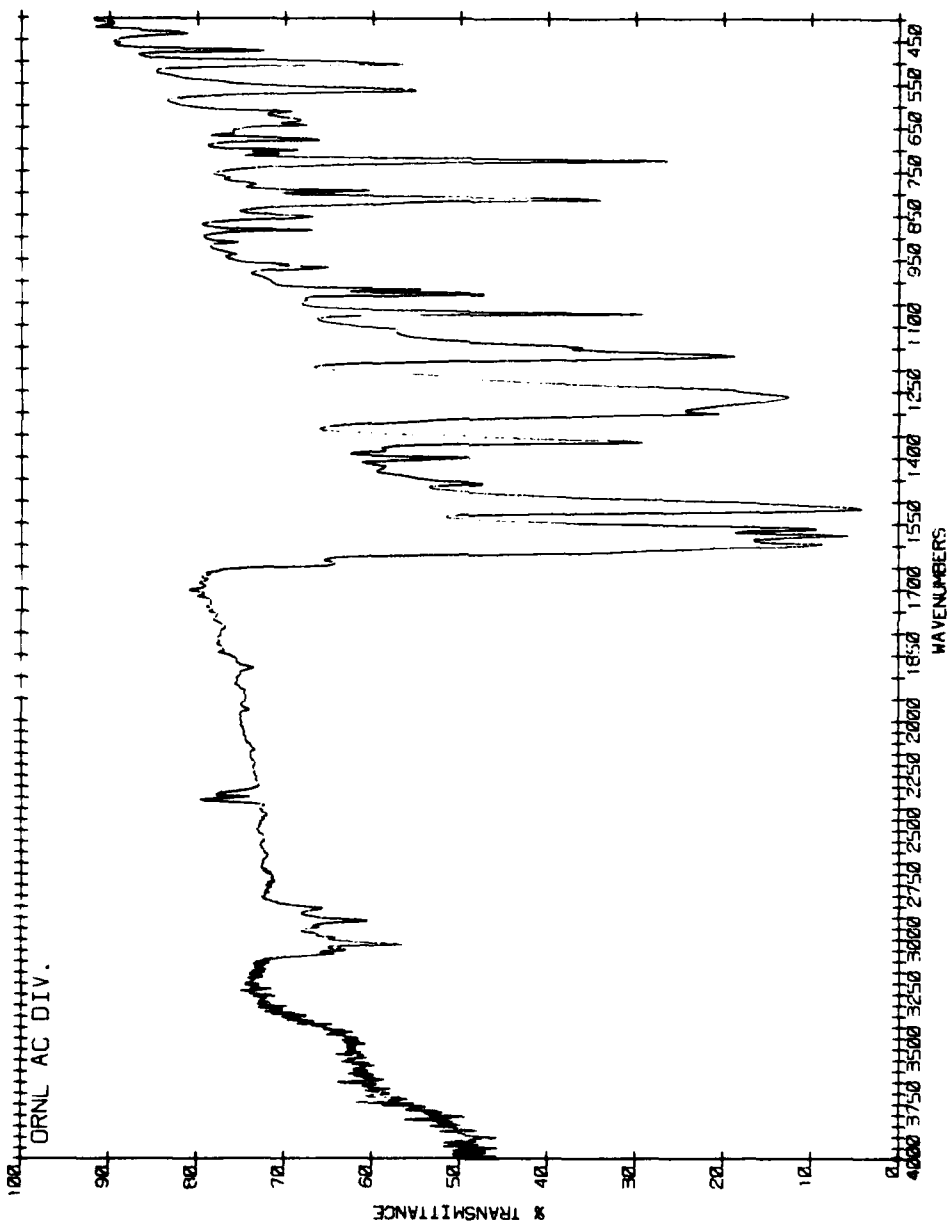

Figure 26. Infrared Spectrum of 1,4-di-p-toluidinoanthraquinone (PTA)

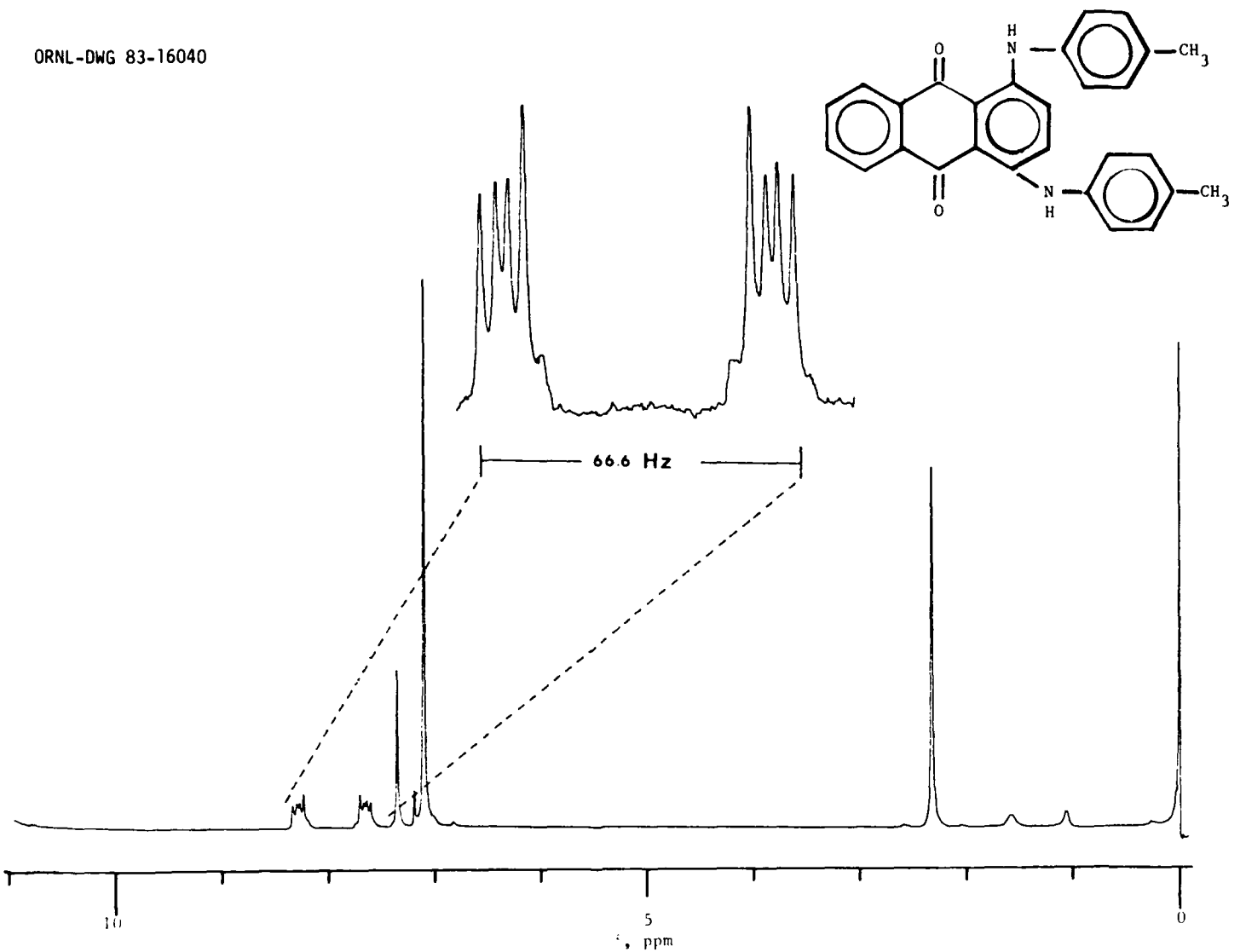

Figure 27. Proton NMR Spectrum of 1,4-di-p-toluidinoanthraquinone (PTA) in $CDCl_3$

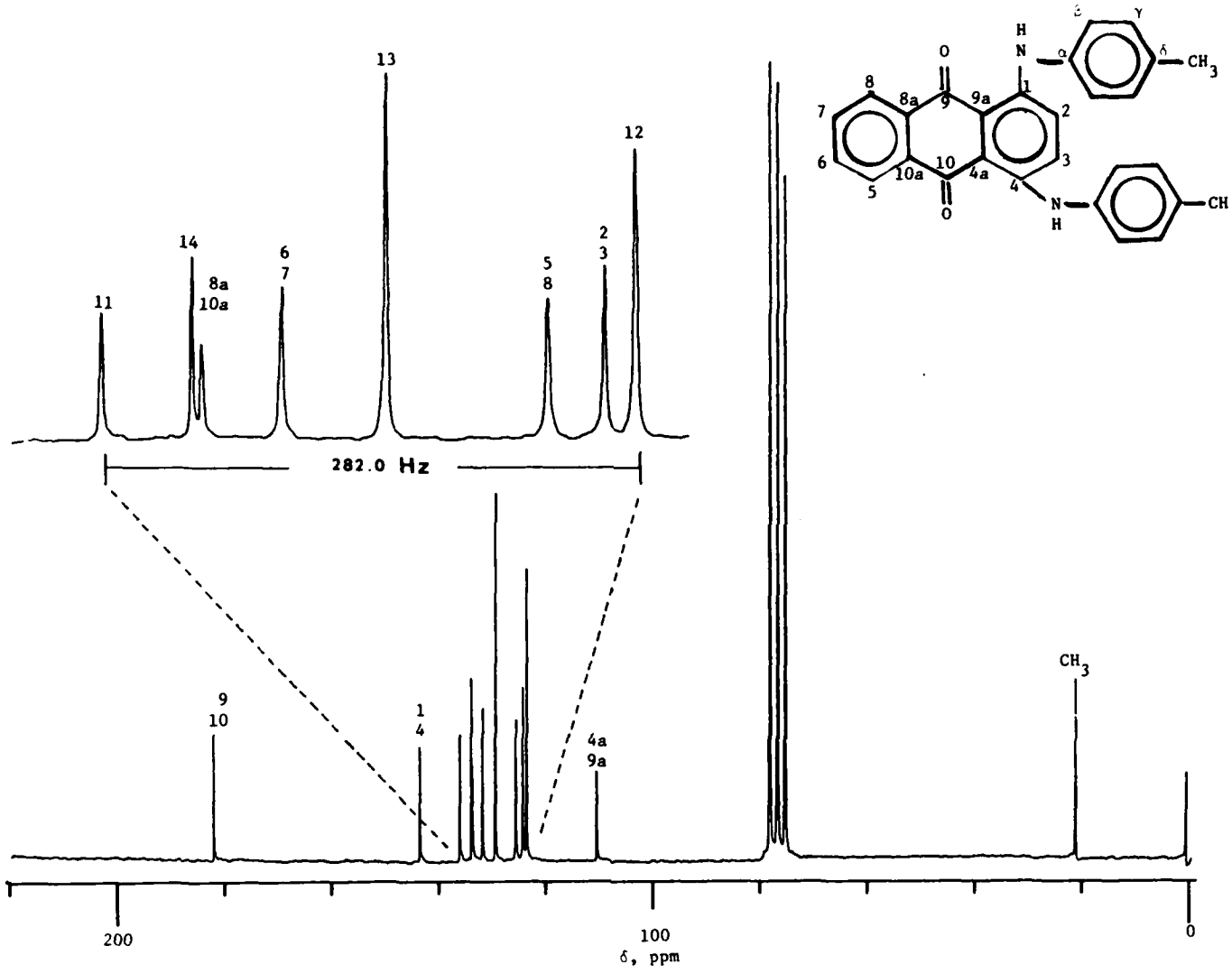

Figure 28. Carbon-13 NMR Spectrum of 1,4-di-p-toluidinoanthraquinone (PTA) in $CDCl_3$

Fraction 1 of the GSM, which was Soxhlet extraction residue, was tested using direct probe mass spectrometry. However, only high molecular weight hydrocarbon-type fragmentations were observed in the resulting mass spectra, with no distinct compounds being identified. These hydrocarbons probably arise from the Marcol 52 antidusting agent as seen previously in the YSM. The second fraction revealed only one compound when studied by TLC, which was thought to be PTA. However, the direct probe MS of this fraction revealed the presence of some BZA, also. The UV/visible, infrared, and ^{13}C NMR spectra of GSM fraction 2 are essentially identical with those of PTA. The TLC of fraction 3 showed that BZA was the major component, along with small amounts of PTA, DBC, and three other impurities. Direct probe MS of this fraction showed only BZA and PTA to be present.

The TLC of fraction 4 showed one large band, corresponding to DBC, and a small amount of two impurities. Only DBC was observed in the direct probe mass spectrum of this fraction. Fraction 5 was found to contain mostly PTA by TLC, along with four smaller bands. The direct probe MS of fraction 5 identified the presence of PTA, BZA, and DBC.

SUMMARY

A summary of the separations and characterizations of the four smoke mixes and the standard dyes is presented in Table 5. The major components identified are listed for each of these materials and include any components present at levels of about ten percent (by weight) or greater. In the case of gas chromatographable materials (primarily the red and violet smoke components), the relative amounts of observed materials are given. For non-volatile materials, the compounds are listed in the approximate order of quantity observed by direct probe mass spectrometry. Minor components (present at less than one percent by weight) are also listed for materials studied by gas chromatography and combined GC/MS. Materials at these trace levels could not be observed by direct probe mass spectrometry in the non-volatile smoke dye components.

The compositions of the five dye standards are also listed in Table 5. The MAA, PTA, and DDA were all greater than 98 percent pure when received. Both the BZA and DBC were fractionated and found to contain a number of impurities, which are listed in Table 5.

TABLE 5. SUMMARY OF CHEMICAL CHARACTERIZATION OF COLORED SMOKE MIXES & DYES PREPARED FOR BIOTESTING

	Wt. % of Whole	Major Components	Minor Components (<1%)[a]
Smoke Mixes			
Red Smoke Mix			
whole		MAA, insolubles (10%)	
fraction 1	12%	MAA (80%), anthraquinone (15%)	aminoanthraquinone, azobenzene, azoxybenzene, aminobiphenyl, phenyldiazobenzene, unidentified ketone (mw = 284)
fraction 2	74%	MAA (>98%)	anthraquinone, 2-methylamino-anthraquinone
fraction 3	13%	non-volatile/non-soluble material	
Violet Smoke Mix			
whole		MAA, DDA, insolubles (24%),	
fraction 1	18%	MAA (>98%)	anthraquinone, DDA, aminonaphthalene, aminoanthraquinone, phenyldiazobenzene, DDA
fraction 2	4%	DDA/DAA[b]	MAA, aminoanthraquinone, aminonaphthalene, anthraquinone
fraction 3	53%	DDA/DAA[b] (95%)	aminonaphthalene
fraction 4	24%	insoluble material	

[a]Observed and identified by GC and GC/MS.

[b]Relative amounts of DDA and DAA were found to vary, see text.

Table 5. (Cont'd)

	Wt. % of Whole	Major Components	Minor Components (< 1%)[a]
Yellow Smoke Mix			
whole		BZA, DBC, insolubles (22%)	
fraction 1	6%	BZA, alkanes	
fraction 2	29%	BZA (>98%)	anthraquinone
fraction 3	11%	BZA (>98%)	
fraction 4	3%	BZA, DBC	ND[c]
fraction 5	5%	BZA, DBC, unidentified ketone (mw = 366)	ND
fraction 6	27%	DBC, unidentified ketone (mw = 366)	ND
fraction 7	22%	DBC and non-volatile material	
Green Smoke Mix			
whole		PTA, BZA, DBC, insolubles (9%)	
fraction 1	9%	alkanes	
fraction 2	62%	PTA, BZA	ND
fraction 3	15%	BZA, PTA, DBC, impurities	ND
fraction 4	4%	DBC, impurities	ND
fraction 5	4%	PTA, BZA, DBC, impurities	ND

[c]ND - Due to solubility and/or volatility limitations, impurities at this level were not detected.

Table 5. (Cont'd)

	Wt. % of Whole	Major Components	Minor Components (< 1%)[a]
Standard Dyes			
MAA		MAA (>98%)	
PTA		PTA (>98%)	
DDA		DDA (>98%)	
BZA		BZA (~80%), insolubles	
fraction 1	11%	BZA (90%) anthraquinone	
fraction 2	54%	BZA	anthraquinone
fraction 3	16%	BZA, black residue	
Residue	19%	non-volatile material	
DBC		DBC, BZA, insolubles (6%)	
fraction 1	13%	alkane, BZA, DBC, diketones (mw = 336 and 366)	ND
fraction 2	12%	BZA, DBC, diketones (mw = 336 and 366)	ND
fraction 3	65%	DBC, dibenzochrysene	ND
Residue	6%	DBC, diketone (mw = 366), involatiles	

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LIST OF ABBREVIATIONS

Sample	Source	Description
BZA	CSL[a]	Benzanthrone standard
MAA	CSL	1-methylaminoanthraquinone standard
PTA	CSL	1,4-di-p-toluidinoanthraquinone standard
DAA		1,4-diaminoanthraquinone
DDA	CSL	1,4-diamino-2,3-dihydroanthraquinone standard
DBC	CSL	dibenzochrysenedione standard
RSM	PBA[b]	Red smoke mix
YSM	PBA	Yellow smoke mix
GSM	PBA	Green smoke mix
VSM	PBA	Violet smoke mix

[a]CSL: Chemical Systems Laboratory, Aberdeen Proving Ground, Maryland 21010

[b]PBA: Pine Bluff Arsenal

PERSONNEL

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M. V. Buchanan
G. Olerich
I. B. Rubin

PUBLICATIONS

The following publications resulted from the work described in this report:

Rubin, I. B., M. V. Buchanan, and G. Olerich. 1982. The Preparative Scale Separation and the Identification of Constituents of Anthraquinone-Derived Dye Mixtures. Part 1. 1-Methylaminoanthraquinone, 1,4-Diamino-2,3-dihydroanthraquinone and 1,4-Diaminoanthraquinone. Anal. Chim. Acta, 135:111-119.

Rubin, I. B., and M. V. Buchanan. 1982. The Preparative Scale Separation and the Identification of Constituents of Anthraquinone-Derived Dye Mixtures. Part 2. Benzanthrone, Dibenzochrysenedione, and 1,4-di-p-Toluidinoanthraquinone. Anal. Chim. Acta, 135:121-128.

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